

Serial No. 09/538,396
Group Art Unit: 1638

REMARKS

Reconsideration and entry of the present amendment after final is respectfully requested.

Status of the Claims:

Claims 2-8, 12, 14, and 18-38 were pending after submission of 13 June 2003. Claims 9-11 have been cancelled in the current amendment, they were previously withdrawn from examination. Applicants reserve the right to pursue the contents of these claims in continuing applications. Claims 2-8, 12, 14, and 18-38 remain under examination after amendment. Claims 12, 14, and 20 have been amended regarding the encoded polypeptide, the phrase "involved in DNA double strand break repair" has been replaced with the phrase "binds to a MRE11 polypeptide". Support for the amendments is found in the claims as originally filed, and throughout the specification, particularly page 1, lines 30-32. No new matter has been added.

Rejections under 35 U.S.C. §112, 1st Paragraph - Enablement:

Claims 2-8, 12, 14, and 18-38 are rejected under 35 U.S.C. §112, first paragraph as failing to comply with the enablement requirement. The rejection is repeated for the reasons of record as set forth in the Office Action of 1/13/03.

The Action asserts, briefly, that given the complexity of DNA repair, including the involvement of other proteins, it is unclear what agronomic benefit a transgenic plant with modulated Rad50 levels would have, and unclear how modulating the level of Rad50 would affect DNA repair or polynucleotide integration. Therefore the Action concludes that one skilled in the art would not know how to use SEQ ID NO: 1, and sequences having 90% and 95% to the disclosed sequences, without undue experimentation.

Applicants respectfully disagree, for the reasons of record (e.g., see response filed 6/13/03), some of which may be reiterated for clarity. Applicants note that

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claims 12, 14, and 20 have been amended, as described above, to recite polypeptides that "bind to a MRE11 polypeptide".

Applicants have provided full-length sequences, demonstrated conserved functional motifs (e.g., page 2, lines 17-24, and Example 4), extensive overall homology, direction regarding nucleotide and amino acid substitutions, and percent identity comparisons (e.g., Appendix A and D; page 5, line 20 – page 6; and line 25; page 16, line 1 – page 20, line 20 of the specification), Rad50 function in DNA break repair and interaction with Mre11 (e.g., page 1, line 15 – page 2, line 14), vector construction, plant transformation, and methods to modulate the level of Rad50 (e.g., page 37, line 5 – page 42, line 22; page 44, line 28 – page 47, line 25; page 47, line 28 – page 49, line 26; page 50, line 29 – page 52, line 31; and page 53, line 1 – page 54, line 6). The Examiner is reminded that the need for routine experimentation and screening is not considered undue (see MPEP 2164.01):

The test of enablement is not whether any experimentation is necessary, but whether, if experimentation is necessary, it is undue. *In re Angstadt*, 190 USPQ 214 (CCPA 1976). An extended period of experimentation may not be undue if the skilled artisan is given sufficient direction or guidance. *In re Colianni*, 195 USPQ 150 (CCPA 1977) (Miller, J., concurring). The experimentation required, in addition to not being undue, must not require ingenuity beyond that expected of one of ordinary skill in the art. *In re Angstadt*, supra. For example, in one instance a "few hours" of experimentation to determine process parameters was not considered to be undue in view of the nature of the invention (preparation of oxygenated hydrocarbons). *In re Borkowski*, 164 USPQ 642 (CCPA 1970). In *Tabuchi v. Nubel*, 194 USPQ 521 (CCPA 1977) a screening procedure which took 15 calendar days was not considered undue experimentation because the test was both simple and straightforward and because of its demonstrated success in producing the desired result.

Further, the Examiner has not given specific reasons, or provided specific citations to support the conclusions that modulation of the level of Rad50 would not have an effect on the plant.

Modulation of Rad50, thereby modulating the level of DNA repair in the plant, is predicted to increase the efficiency of incorporation of heterologous nucleic acids

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in the genome of a plant. For the record, Applicants disagree with the Examiner's conclusion that modulation of Rad50 must result in an agronomic benefit to the transgenic plant. Applicants utility, *to use Rad50 to modulate the efficiency of incorporation of a transgene into the plant genome* (page 2, lines 10-14) does not require that Rad50 confer any particular agronomic phenotype to the plant.

Applicants note that they have provided another component of the DNA repair complex, Mre11, in U.S. Patent 6,646,182, issued Nov. 11, 2003, thereby demonstrating the presence of Mre11 in plants. Also, Gallego *et al.* have demonstrated Rad50 involvement in DNA repair and meiosis in *Arabidopsis* (*Plant J.* 25:31-41 2001, submitted in Appendix C). Applicants have modified the Multiple Sequence Alignment (Appendix A) to include the conserved motifs shown in Example 4 of the specification, currently presented as Appendix D. Applicants note that the alignment is similar to the one shown by Gallego *et al.* (*supra*), which shows higher conservation of the N- and C-terminal regions of Rad50. Appendix D also includes the results of an analysis performed using the Lion BioScout software package, and Hmmerpfam search using GCG software, both of which detect the known Rad50 Zn-hook Pfam domain ($e = 6.9e-07$).

Applicants assert this support fully enables one of skill in the art to make and use the full-breadth of the invention without undue experimentation. Absent of any evidence from the Examiner supporting the conclusion that modulation of Rad50 would not effect the plant, Applicants believe this rejection cannot be supported. Therefore Applicants respectfully request reconsideration and that the rejection of claims 2-8, 12, 14, and 18-38 under 35 U.S.C. §112, first paragraph for lack of enablement be withdrawn.

Rejections under 35 U.S.C. §112, 1st Paragraph, Written Description:

Claims 2-8, 12, 14, 20 and 23-38 are rejected under 35 U.S.C. §112, first paragraph, as containing subject matter not sufficiently described in the specification

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to indicate the inventor(s) had possession of the invention. The rejection is repeated for the reasons of record as set forth in the Office Action of 1/13/03.

Briefly, the Action asserts that the Applicant has not described a representative sample of the genus, and that the conserved domains are not unique to Rad50 proteins, but are common to all DNA repair proteins. The Action also asserts that Example 14 of the Revised Interim Written Description Guidelines is not applicable because claims drawn to polynucleotides having 95% sequence identity are not rejected in this Action.

Applicants respectfully disagree, for the reasons of record (e.g., see response filed 6/13/03), some of which may be reiterated for clarity. As is stated in the MPEP 2163 (see p. 2100-168) the written description for a claimed genus may be satisfied by "disclosure of relevant, identifying characteristics, i.e., structure or other physical and/or chemical properties, by functional characteristics coupled with known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show that the applicant was in possession of the claimed genus." Applicants respectfully submit that the present application meets this standard by the disclosure of the structures of the full-length Rad50 polynucleotide (SEQ ID NO: 1), which encodes a full-length Rad50 polypeptide (SEQ ID NO: 2) which have the structural/chemical properties of significant sequence identity to known Rad50 polynucleotides and polypeptides, conserved domains (see, for example, Example 4 and the Multiple Sequence Alignment), and the functional characteristic of Rad50 polypeptide binding to MRE11 polypeptides.

Regarding the applicability of Example 14, for the record Applicant notes that while claims 18 and 21, directed to sequences having 95% identity to either SEQ ID NO: 1 or SEQ ID NO: 2, are not included in this rejection, they are also not allowed. Also, the Examiner has not made clear why Example 14 does not apply, other than focusing on the specific percent identity recited and not the substantive features of the structural and functional elements. Applicants maintain that the current claims, including those directed to 90% sequence identity, meet the Written Description

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standard as set forth in the 35 U.S.C. §112, described MPEP, and shown in Example 14 of the Revised Interim Written Description Guidelines.

Applicants have coupled structural, chemical, and functional properties to describe polynucleotides having 90% and 95% sequence identity to SEQ ID NO: 1, or encoding polypeptides that have 90% and 95% sequence identity to SEQ ID NO: 2 such that a person skilled in the art can envisage the claimed invention, thereby meeting the 35 U.S.C. §112, first paragraph written description requirement. Therefore Applicants respectfully request reconsideration and that the rejection of claims 2-8, 12, 14, 20 and 23-38 under 35 U.S.C. §112, first paragraph for lack of written description be withdrawn.

CONCLUSION

In light of the foregoing remarks and amendments, it is believed that claims 2-8, 12, 14 and 18-38 are in condition for allowance. Withdrawal of the outstanding rejections and allowance of all of the remaining claims is respectfully requested. The Examiner is invited to telephone the Applicants representative to expedite prosecution and allowance.

Respectfully submitted,



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APPENDIX C

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Disruption of the *Arabidopsis RAD50* gene leads to plant sterility and MMS sensitivity

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Summary

The Rad50 protein is involved in the cellular response to DNA-double strand breaks (DSBs), including the detection of damage, activation of cell-cycle checkpoints, and DSB repair via recombination. It is essential for meiosis in yeast, is involved in telomere maintenance, and is essential for cellular viability in mice. Here we present the isolation, sequence and characterization of the *Arabidopsis thaliana RAD50* homologue (*AtRAD50*) and an *Arabidopsis* mutant of this gene. A single copy of this gene is present in the *Arabidopsis* genome, located on chromosome II. Northern analysis shows a single 4.3 Kb mRNA species in all plant tissues tested, which is strongly enriched in flowers and other tissues with many dividing cells. The predicted protein presents strong conservation with the other known Rad50 homologues of the amino- and carboxy-terminal regions. Mutant plants present a sterility phenotype which co-segregates with the T-DNA insertion. Molecular analysis of the mutant plants shows that the sterility phenotype is present only in the plants homozygous for the T-DNA insertion. An *in vitro* mutant cell line, derived from the mutant plant, shows a clear hypersensitivity to the DNA-damaging agent methylmethane sulphonate, suggesting a role of *RAD50* in double-strand break repair in plant cells. This is the first report of a plant mutated in a protein of the Rad50–Mre11–Xrs2 complex, as well as the first data suggesting the involvement of the Rad50 homologue protein in meiosis and DNA repair in plants.

Keywords: *Arabidopsis*, *RAD50*, DSB repair, sterility, recombination.

Introduction

The repair of DNA double-strand breaks (DSBs) involves genetic recombination. Fundamentally, two different forms of recombination are involved: those involving DNA sequence homology between the participating DNA molecules, and those that appear to act independently of such homology, called homologous recombination (HR) and non-homologous end-joining (NHEJ), respectively. Considerable advances in the understanding of these mechanisms have been made in recent years, both in terms of the proteins involved and of their relative contribution to repair of DSBs in different organisms and/or cell types. These issues are the subject of a number of recent reviews (Fox and Smith, 1998; Jeggo, 1998; Paques and Haber, 1999; Petrini *et al.*, 1997; Roeder, 1997; Smith, 1998; Smith and Nicolas, 1998; Tsukamoto and Ikeda, 1998).

Much of our understanding of the proteins implicated in the processes of recombination and DSB repair comes from studies of the yeast *Saccharomyces cerevisiae*. Homologous recombination in yeast cells is largely under the control of the genes in the *RAD52* epistasis group, which include *RAD50–59*, *XRS2* and *MRE11* (Ajimura *et al.*, 1993; Game, 1993; Haynes and Kunz, 1981). These genes were mostly identified as being needed for the repair of ionizing radiation-induced DNA damage, and are also needed for meiosis. Deletion of these genes in yeast generates defects in both recombination and DNA repair with differing phenotypes in terms of recombination and the repair of DNA double-strand breaks. Mutants of *rad51*, *rad52*, *rad54*, *rad55*, *rad57* and *rad59* show defects in HR, while *rad50* and *mre11* mutants have shown defects in NHEJ and are hyper-rec for HR.

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Double-strand breaks are repaired by both HR and NHEJ in all cell types examined; however, the preferred mechanism of recombinational DNA repair differs significantly in a given organism. In particular, it is clear that non-homologous recombination is much more frequent than homologous recombination in mammalian and plant cells, whereas yeast cells rely almost entirely on homology-based recombinational DNA repair. The basis of the strong preference for illegitimate recombination in mammalian and plant cells is not fully understood. Both pathways have a common initial substrate (DSB), the processing of which may be channelled into either HR or NHEJ. This channeling is apparently determined by the initial metabolism of the DSB itself, and it has recently been suggested that it may result from a competition for binding the DNA ends between the Ku and Rad52 proteins (VanDyck *et al.*, 1999). In this context, the Rad50/Mre11/Xrs2 complex is of particular interest, as mutants show a weak hyper-HR and strong hypo-NHEJ phenotype in mitotic yeast cells (Boulton and Jackson, 1998; Moore and Haber, 1996; Schiestl *et al.*, 1994; Tsukamoto *et al.*, 1996; Tsukamoto *et al.*, 1997). However, in some assays RAD50 is needed for intrachromosomal recombination (Elias-Arnanz *et al.*, 1996; Rattray and Symington, 1996; Tran *et al.*, 1995). Rad50 mutants show altered processing of DNA ends during recombination (Ivanov *et al.*, 1994; Sugawara and Haber, 1992). This complex has also been implicated in the regulation of the response of yeast cells to DNA damage (Kromani and Muniyappa, 1997; Lee *et al.*, 1998).

Homologues of many of these yeast genes have been cloned from different organisms (see reviews by Kanaar and Hoeijmakers, 1997; Paques and Haber, 1999), including RAD50 genes from *S. cerevisiae* (Alani *et al.*, 1989); *S. pombe* (Saunders, 1999); man (Dolganov *et al.*, 1996); mouse (Kim *et al.*, 1996); and *Caenorhabditis elegans* (Offenberg and Heyting, 1996). Rad50 shows homology to the *Escherichia coli* SbcC protein (Sharples and Leach, 1995), and has been shown to form a complex with the Mre11, Xrs2 and Lig4 proteins. The Rad50/Mre11 complex is involved in DSB repair via NHEJ in mammalian cells, and the null mouse *rad50* mutant is inviable both in cultured ES cells and in developing mouse embryos (Luo *et al.*, 1999). This cell-lethal phenotype has also been seen in mouse *mre11* mutants (Xiao and Weaver, 1997), as well as chicken (Sonoda *et al.*, 1998) and mouse *rad51* mutants (Lim and Hasty, 1996; Sharan *et al.*, 1997; Tsuzuki *et al.*, 1996). Interestingly, *rad52* mutant mice are viable and show increased radioresistance (Rijkers *et al.*, 1998; Yamaguchi-Iwai *et al.*, 1998).

A number of radiation-sensitive plant mutants have been isolated in recent years (reviewed by Britt, 1999; Gorbunova and Levy, 1999; Mengiste and Paszkowski, 1999). The mutated genes of methylmethane sulphonate (MMS) and UV-hypersensitive *Arabidopsis* mutants have

recently been identified: a ribosomal S27 protein (Revenkova *et al.*, 1999); the *Arabidopsis* Rad1 homologue protein (Gallego *et al.*, 2000; Liu *et al.*, 2000); and a member of the structural maintenance of chromosomes protein family (SMC; Mengiste *et al.*, 1999; Strunnikov, 1998; Strunnikov *et al.*, 1993). Rad50 is also a member of this family. Plant homologues of RAD51 (Doutriaux *et al.*, 1998; Smith *et al.*, 1996) and MRE11 (Hartung and Puchta, 1999) have been isolated, and the rapid progress of the *Arabidopsis* sequencing project makes it likely that many other genes implicated in these processes will be isolated in the near future.

As part of our investigation of the control of the early events of DSB repair and recombination in plants, we have isolated the *Arabidopsis thaliana* homologue of the RAD50 gene, and here we describe the isolation, sequencing and preliminary characterization of this gene. Furthermore, we have identified a *rad50* mutant plant that presents a sterility phenotype in agreement with the role of this protein in meiosis in yeast cells. We also show that homozygous mutant cell lines present hypersensitivity to MMS, suggesting (as has been shown in yeast cells) a role for RAD50 in DSB repair in plants. This is the first evidence of the implication of the RAD50 gene in meiosis as well as in DSB repair in plants.

Results and Discussion

The *Arabidopsis thaliana* RAD50 homologue

A 700 bp *A. thaliana* cDNA clone with homology to the carboxy-terminal region of the yeast *Rad50* gene was detected in a screen of the *Arabidopsis* cDNA expression library with an antibody against a peptide sequence from the cytoplasmic domain of vertebrate beta 1 integrin. Based on this sequence (a kind gift from P. Nagpal and R. Quatrano), oligonucleotides were designed and a fragment of 1.5 kb of genomic sequence was amplified from genomic DNA prepared from an *Arabidopsis* cell-suspension culture. This DNA fragment was used to screen a genomic lambda bank prepared with DNA from the same cells, permitting the identification of two lambda clones with overlapping inserts spanning approximately 22 kb of genomic DNA and including the *Arabidopsis* RAD50 homologue locus. Sequencing of 10 kb from the inserts of these clones showed that they spanned the entire *AtRAD50* gene (data not shown). Our genomic DNA sequence has recently been confirmed by (and is identical to that of) the *Arabidopsis* genome sequencing project (Lin *et al.*, 1999).

The cDNA encoding the *Arabidopsis* RAD50 homologue was isolated by RACE-PCR with the Marathon Kit (Clontech Inc., Palo Alto, CA, USA). Poly(A)⁺ RNA was prepared from total RNA isolated from the *A. thaliana* cell

suspension; double-stranded cDNA was synthesized and adapters were ligated to the two ends. This library of adapter-ligated cDNA was PCR amplified using a *RAD50* specific primer and primers against the adapter sequences. A PCR fragment of 4.3 kb was isolated on agarose gel and cloned into pGEM-Teasy (Promega Inc., Madison, WI, USA). Sequencing of the 4305 bp insert clone confirmed that it includes the entire *AtRAD50* cDNA, and that this cDNA corresponds to the genomic clones (above). The gene covers 8486 bp and contains 27 exons; all 5' donor sites contain the AG/GT conserved junction; and the conserved AG is present in all the 3' acceptor sites. We have submitted this mRNA sequence to GenBank (Gallego *et al.*, 1999). The computer prediction of the mRNA sequence of this gene from the genome sequencing project (Lin *et al.*, 1999) misplaces a number of the intron-exon borders, and thus predicts a protein with 53 inserted and 32 deleted amino acids relative to that predicted from our cDNA sequence.

Amino acid comparison of Rad50 homologues.

The cloned cDNA from *Arabidopsis* presents a putative methionine initiation codon at nucleotide residue 146 in the first exon. No other open reading frames were detected. The termination codon of the open reading frame is located at nucleotides in position 4097, giving a predicted protein of 1316 amino acids. A 3'-untranslated region covers 181 nt before the short poly(A) tail present in the cDNA clone. The length of the protein is strongly conserved in all organisms in which it has been studied: the human, mouse and yeast Rad50 proteins have 1312 amino acids, while the *C. elegans* Rad50 has 1298 and the *Arabidopsis* *RAD50* cDNA reported here predicts a protein of 1316 amino acids. Amino acid sequence comparison with the known Rad50 proteins shows a high conservation at the N- and C-terminal regions. In particular, the amino-terminal 190 amino acid region of the *Arabidopsis* protein has 56 and 52% sequence identity with the yeast and human Rad50 proteins, respectively. The carboxy-terminal 207 amino acids show >62 and 52% identity with the corresponding region present in the yeast and human Rad50 proteins, respectively (Figure 1a). The overall amino acid identity of the predicted *Arabidopsis* protein is of 29.8% with the human and 27.3% with the yeast proteins, respectively. The *Arabidopsis* Rad50 protein predicted from the cDNA sequence contains 18.2% of acidic amino acids and 16.9% of basic residues. The protein is predominantly hydrophilic, with 30.3% of hydrophobic residues. It presents three glycosylation motifs in positions 398–399, 419–422 and 620–623. A type-A ATP-binding site is present in the amino-terminal region at amino acid positions 34–41. A Walker B motif is present at the carboxy-terminal region between amino acids 1235 and 1242. Checking of

the predicted *AtRad50* amino acid sequence for plant localization signals with the PSORT program (<http://psort.nibb.ac.jp/>), yields a putative nuclear localization signal with certainty of 0.350 (success). No chloroplast or mitochondrial localization sequences were identified in the sequence by PSORT. Figure 1(b) shows the output from the COILS program (http://ulrec3.unil.ch/software/coils_FORM.html; Lupas *et al.*, 1991). It can be seen that the predicted *AtRad50* protein conserves the central coiled-coiled conformation domain seen in the yeast and human proteins (Dolganov *et al.*, 1996).

Chromosomal localization of Arabidopsis RAD50 locus

Southern analysis of DNA prepared from *Arabidopsis* suspension cells show a unique band after digestion with six different restriction enzymes (Figure 2a), suggesting that the *Arabidopsis* *RAD50* gene is present as a single-copy gene within the *A. thaliana* genome.

Using PCR amplification of DNA prepared from the *Arabidopsis* YAC bank (Creusot *et al.*, 1995), we have mapped the *RAD50* gene on chromosome II (YAC C1C 11E1L) near the marker *TEN5* (data not shown). Recently, genomic sequencing results of *Arabidopsis* chromosome II BAC F22D22 has confirmed this mapping (Lin *et al.*, 1999).

Expression of the RAD50 gene in Arabidopsis

Northern analysis of RNAs prepared from *Arabidopsis* suspension cells as well as different plant tissues shows a single mRNA species, the length of which corresponds to that of the cDNA (Figure 2b). This has been confirmed by Northern analysis of poly(A)⁺ RNA from suspension cells and flower buds, as well as by RT-PCR analysis (data not shown).

Studies of human and mouse *RAD50* gene expression have shown the presence of alternative spliced transcripts (Kim *et al.*, 1998; Kim *et al.*, 1999). We were unable to detect alternative transcripts in *A. thaliana* using both Northern and PCR analysis. The *Arabidopsis* *RAD50* mRNA is expressed in all cell tissues analysed; however, stronger levels were found in fast growing cells such as cell-suspension culture, young primary roots and flowering structures. Tissue from flower buds and mature flowers contains a relatively high proportion of cells undergoing division, processes that appear to be correlated with a high level of expression of *RAD50*. Maximum expression of human and mouse *RAD50* genes has been detected in the testis (Dolganov *et al.*, 1996; Kim *et al.*, 1996). Thus, although the transcript is enriched in meiotic tissues, expression of the *AtRAD50* gene is not specific to meiotic cells. This pattern of expression is reminiscent of that previously reported for the *Arabidopsis* *RAD51* homologue

(Doutriaux *et al.*, 1998) and those of the *UVH1/AtRAD1* (Gallego *et al.*, 2000; Liu *et al.*, 2000) and *UVR2* (Ahmad *et al.*, 1997) genes of *Arabidopsis*.

As reported for the human Rad50 protein following treatment with ionizing radiation (Dolganov *et al.*, 1996), the steady-state level of *AtRAD50* mRNA does not change in cultured cells in response to exposure to the radio-mimetic compound MMS (data not shown). This contrasts with the situation reported for mouse *RAD50* gene expression, which does increase after treatment of NIH-3T3 cells with MMS (Kim *et al.*, 1996).

Identification and characterization of a *rad50* mutant of *Arabidopsis*

Based on our *AtRAD50* sequence, we designed oligonucleotides and screened the Versailles *Arabidopsis* T-DNA insertion mutant collection (Bechtold *et al.*, 1993; Bouchez *et al.*, 1993) using PCR as described by Gaellen *et al.* (2000). From this screen we identified a single plant containing a T-DNA insertion in the *RAD50* locus. The mutant plant is fully sterile, producing numerous flowers and small, empty siliques (Figure 3).

The mutant phenotype and the kanamycin resistance marker of the inserted T-DNA co-segregate, with selfed heterozygotes producing progeny with the 3:1 ratio expected for a single-locus insertion (128 non-mutant:38 mutant; χ^2 , 1 df = 0.39). Southern analysis has confirmed that the T-DNA insertion is present at a single locus (not shown), and PCR results indicate the single-copy nature of the insertion (see the size of the amplified fragment present in the homozygous plants, Figure 4). Molecular analysis shows that the 38 plants presenting the sterility

phenotype are, as expected, homozygous for the T-DNA insertion. Thus there is clear linkage between the T-DNA insertion and the sterility phenotype of the mutant *rad50* allele, which is recessive. Furthermore, we have also observed a sterility phenotype in plants expressing *AtRAD50* antisense RNA (not shown). These results suggest a role for the Rad50 protein in meiosis in plants which is consistent with that known in yeast. Due to the cell-lethal phenotype of the *rad50* null mutant in mouse, this constitutes the first implication of *RAD50* in meiosis in a metazoan organism.

Mutant plants which have been germinated on agar medium before being transferred to soil are reduced in size in comparison to their non-mutant siblings; however this

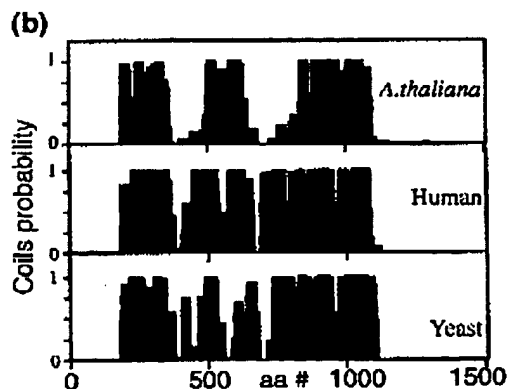


Figure 1b. Legend on facing page.

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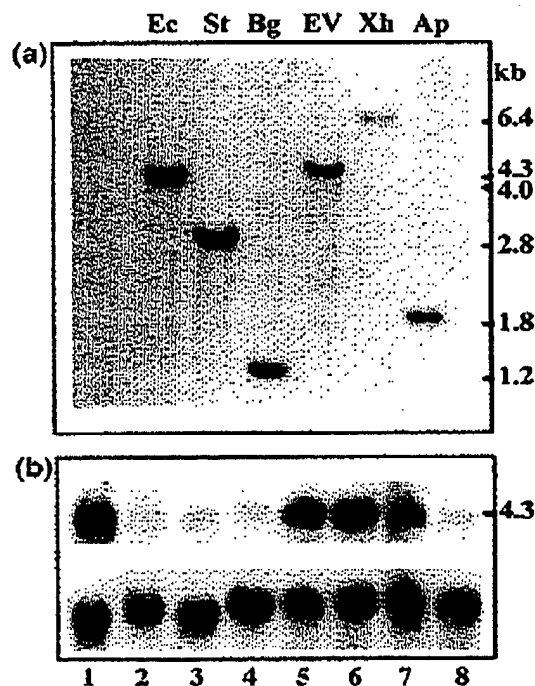


Figure 2. Southern and Northern analysis of *AtRAD50*. (a) Southern analysis of DNA from *Arabidopsis* cell culture probed with the *AtRAD50* genomic probe. From left to right: *EcoRI*, *StyI*, *BglII*, *EcoRV*, *XhoI* and *AclI*-digested DNA. Sizes of the detected fragments are shown to the left. (b) Northern analysis of total *Arabidopsis* RNA from different tissues, probed with the 3' half of the *AtRAD50* cDNA (upper) and the 18S rDNA probe as a loading control (lower). Lanes: 1, suspension cells; 2, young rosette leaves grown for 3 weeks; 3, leaves from stems of mature flowering plants; 4, rosette leaves from non-flowering plants grown for 6 weeks with short daylength (8 h light period); 5, mature flowers; 6, flower buds; 7, roots; 8, stems.

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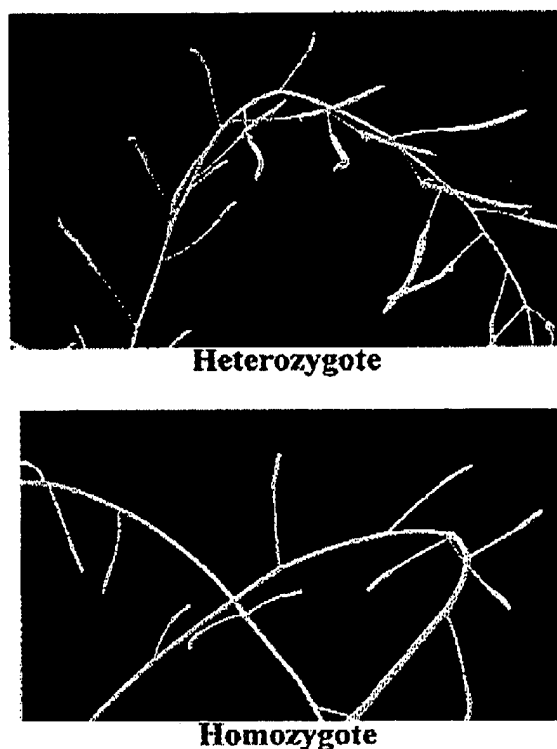


Figure 3. The *rad50* mutant is sterile. Photographs of 7-week-old mutant (*rad50/rad50*) and heterozygote (*rad50/RAD50*) plants. The mutant plants produce many flowers and small, empty siliques.

phenotype is not seen if seeds are germinated directly in soil in the greenhouse. This growth difference is seen whether or not antibiotic (kanamycin) is included in the agar medium, and is thus not an artefact caused by the antibiotic. Further studies will be needed to determine the exact origin of this facultative growth defect.

The mutated *rad50* gene and its inserted T-DNA were PCR-amplified, cloned, and the plant DNA/T-DNA junctions sequenced to determine the exact position and structure of the insertion (Figure 5). The T-DNA is inserted into the coding sequence of the gene and causes disruption of the *RAD50* ORF in the 21st exon, after the 1050th codon (of 1316). Thus any mutant protein produced would lack the 266 C-terminal amino acids of the wild-type protein.

The inserted T-DNA has a small, 141 nucleotide, inverted duplication of the LB sequence upstream of the RB, and has lost the first 10 nucleotides following the RB nick site. The inverted orientation of this LB DNA implies the participation of a replicated T-DNA molecule in the origin of this sequence, and that it is not simply the result of

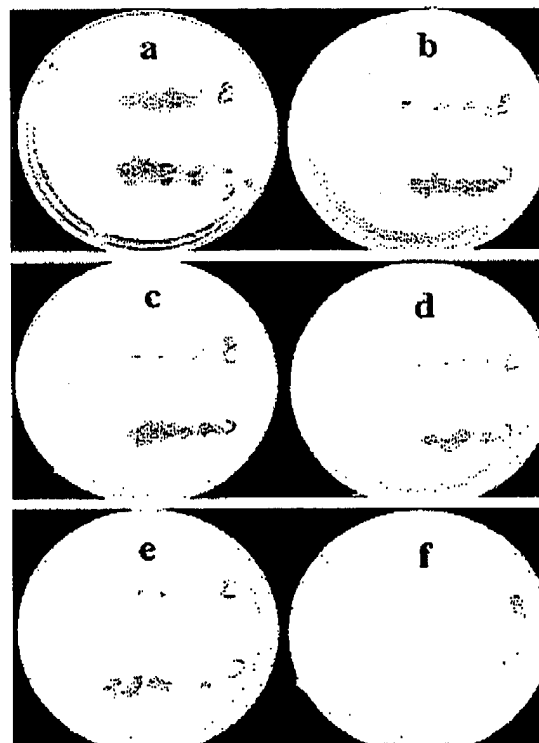


Figure 6. MMS sensitivity of *rad50* mutant cells. Mutant (*rad50/rad50* upper on each plate) and heterozygote (*RAD50/rad50* lower on each plate) suspension cell lines grown in Petri dishes on agar medium containing different concentrations of MMS: (a) no MMS; (b) 0.0033%; (c) 0.0067%; (d) 0.01%; (e) 0.0133%; (f) 0.0167% v/v MMS.

ligation-derived concatamers of the single-stranded transforming T-DNA molecules. The beginning of the insertion (inverted LB sequence) has a 12 bp (with three mismatches) homology to the *RAD50* sequence at that position. The LB junction is much simpler, with the T-DNA sequence ending 18 nt downstream of the LB nick site, followed by the insertion of two As prior to resumption of the *RAD50* gene sequence. The insertion caused an 18 bp deletion (not including the 12 bp homology, above) of *RAD50* sequence. Neither of the two junctions occurs exactly at the nick site of the corresponding border sequence. We have previously seen similar structures at T-DNA insertion sites (unpublished results), and the mechanism of T-DNA integration has been reviewed by Tinland (1996). The systematic sequencing of the DNA flanking T-DNA insertions of the Versailles mutant collection is currently under way, and understanding of these events will greatly benefit from this rich source of data.

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The Arabidopsis rad50 mutant is hypersensitive to MMS

In yeast cells, mutation of the *RAD50* gene causes hypersensitivity to X-ray irradiation and to particular radio-mimetic DNA-damaging chemicals such as MMS. However, due to the cell-lethality of *rad50* mutants in

mouse, a clear demonstration of this radiosensitivity has not previously been possible in a metazoan organism (see Luo *et al.*, 1999 for work on gamma-irradiated early mouse blastocyst explants).

We and colleagues (J. Paszkowski, personal communication) have observed that the lethal effects of MMS on *Arabidopsis* seedlings are dependent on the size of the plants tested. Non-ambiguous determination of the MMS concentration needed to kill a given plant line (or other multicellular organism) is thus complicated by questions of the size of plants used. In order to avoid this problem, we therefore tested MMS sensitivity on cell lines derived from these plants. In this way, very small clumps (microcalluses) of growing cells are exposed to MMS, and a much clearer determination of dose-dependent MMS sensitivity is possible. Callus was induced on young leaf tissue, and suspension cultures initiated using standard protocols (see Experimental procedures). Suspension cells in liquid culture were pipetted onto solid growth media with or without different concentrations of MMS, and their growth scored visually 2–3 weeks later (Figure 6). In the absence of MMS the *rad50* mutant and heterozygote cell lines had both grown to similar extents, whilst the *rad50* mutant cells showed a clear hypersensitivity to MMS relative to the heterozygote cell line. We have also observed MMS sensitivity in mutant cell lines generated via the antisense approach (data not shown). These data suggest that the Rad50 protein plays a role in DSB repair in plant cells, as is the case in yeast.

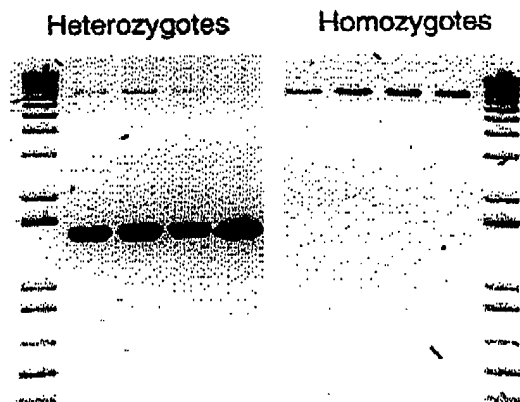


Figure 4. The *rad50* mutant locus contains a single T-DNA insertion. PCR of individual plants segregating *RAD50*. Total DNA was amplified with a pair of primers in the *RAD50* sequence, spanning the T-DNA insertion site (5'-GAGCTGTGAAGCTAGAAAGAATGAACCTTGCAAGGTG; 5'-CCCATCCAGGTTTGTAGTTG). Homozygote plants show only the 8 kb PCR product expected for a single-copy T-DNA insertion, while heterozygotes also show the expected 1.5 kb band for the wild-type *RAD50* gene.

| | |
|---|--|
| (a) Right border junction | |
| MUTANT | AAAGTTGAGGAGCTTACACGTAAAAATGAGGCAATCGATTCTACTGAATCCGATTTCAGGGTCTGGCCAAAACCTATTCCGT |
| LB-RB | tgccaggatataatgccaaCGTAAAAATGAGGCAATCGATTCTACTGAATCCGATTTCAGGGTCTGGCCAAAACCTATTCCGT |
| RAD50 | AAAGTTGAGGAGCTTACACCTGagattgaatcattggagagcaaat |
| (b) Left border junction | |
| MUTANT | GGGCACCTGGCACACGCCCTGGAGTCCGCCCGCTTCCAGTTGAGGGTTGTCTACGCTTAGATGACAAACAACTTCTTAAACG |
| LB-RB | GGGCACCTGGCACACGCCCTGGAGTCCGCCCGCTTCCAGTTGAGGGTTGTCTACGCTTAGATGAGAAAGGAAAGTTGTCCBaga |
| RB-LB | acacgattttattTAAACG |
| (c) Mutant protein | |
| TAC CGG ACA ACA AAA GCA AAA GTT GAG GAG CTT ACA CGT | AAA AAT GAG GGC AAT CGA TTG TAC TGA ATC |
| X R T T K A K V E E L T R | K N E G N R L Y * |
| aa1050 | |

Figure 5. Sequences of junctions of the inserted T-DNA.

RAD50 junctions (a,b) and the predicted effect on the Rad50 protein (c). The mutant *rad50* genomic DNA sequence is aligned with the sequences of the ends of the T-DNA used to generate the *Arabidopsis* mutants, and with the *RAD50* genomic sequence. *RAD50* sequence deleted at the point of the insertion is boxed (a) and the RB-LB sequence shown begins immediately after the RB nick (b). In frame coding DNA (upper) and protein (lower) sequence added to the mutant *rad50* open reading frame by the insertion is shown boxed in (c).

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The Arabidopsis rad50 mutant is viable

To our knowledge, the only other report of a *rad50* mutant in a metazoan organism describes a 'cell-lethal' phenotype in a mouse *rad50* mutant (Luo *et al.*, 1999). These authors were unable to isolate homozygous *rad50* mutants of mouse ES cells in culture, and ascribed the early embryonic lethality of homozygous *rad50/rad50* mice to a gradual cession of proliferation of the embryonic cells. A differential radiosensitivity was also seen in mutant blastocyst explants, and the authors tentatively concluded that the cell lethality is caused by the accumulation of unrepaired and/or misrepaired DNA DSBs, indicating involvement of mouse Rad50 protein in DSB repair/signalling.

We show here that the *Arabidopsis rad50* mutant is hypersensitive to the radiomimetic agent MMS, and thus presumably defective in DSB repair; however we have no evidence for a strong effect on the proliferative capacity of *rad50* *Arabidopsis* cells. Under certain growth conditions the mutant plant is stunted, but the facultative nature of this phenotype clearly indicates the lack of a basic problem at the cell division level. As the mutant plant is fully sterile, effects on cellular proliferative capacity can only be studied in the context of a single plant generation. Thus we have derived mitotic cell cultures from the mutant plants in order to be able to study long-term effects. These cells grow, and we are undertaking further analysis on the involvement of the *RAD50* function in DNA repair/recombination/cell proliferation in plant cells.

As the *rad50* mutation in our mutant is caused by a DNA insertion into the coding sequence of the *AtRAD50* gene, we cannot exclude that a truncated protein is produced in the mutant cells, which would thus not be true 'null' mutants. Preliminary data using antibodies to a C-terminal fragment of the AtRad50 protein shows the absence of the AtRad50 protein band on Western blots of protein from the mutant cells (S. Daoudal and C.I.W., unpublished results); however these antibodies may not recognize a C-terminus-truncated form of the protein. It is thus formally possible that this putative truncated protein is sufficient to fulfil the function of the Rad50 protein in cell viability. However, given the clear phenotypes of the mutant for meiosis and MMS sensitivity, we think it extremely unlikely that the viability of the *rad50* mutant cells and plants can be explained in this way. We note that the question of whether a given mutation is null may be posed for any insertional mutation that does not involve full deletion of the open reading frame of the gene in question (i.e. including all insertional mutations). Whether or not our mutant is 'null' does not alter our conclusions on the role of the AtRad50 protein in *Arabidopsis* and the phenotypes of the T-DNA insertion mutant described here. Full reso-

lution of this question must await the identification of a full *AtRAD50* deletion mutant.

In conclusion, the plant Rad50 function appears to be analogous to that of yeast. Given the difficulties encountered in isolating and studying the mouse *rad50* mutant, we believe that further study of *rad50* mutant plant cells will permit us to clarify the role of this polyvalent protein and its multiple roles in the maintenance of genome integrity in both plants and animals.

Experimental procedures

Growth of cell suspensions and plants

The *Arabidopsis thaliana* cell suspension (TB7) was established by Axelos *et al.* (1992). Cells were grown in Gamborg's B-5 medium (Sigma #G5893, St Quentin Fallavier, France) supplemented with 30 g l⁻¹ sucrose and 200 mg l⁻¹ naphthalene acetic acid (NAA) on a rotating platform (120 rpm) at 22°C with 16 h light/8 h dark. Cells in liquid culture were subcultured at weekly intervals. Bacto-agar (Difco, Detroit, MI, USA) was included in the cell-suspension medium at 0.8% w/v for solid media. *Arabidopsis thaliana* (Columbia) seeds were sown directly into damp compost and germinated in a greenhouse under white light (16 h light/8 h dark). For the isolation of root tissue, plants were grown aeroponically under the same conditions.

Callus cultures were derived from homozygous *rad50/rad50* and heterozygous *rad50/RAD50* plants using standard techniques as follows (J. Lucht and B. Hohn, personal communication). Leaves of young germinating plants were surface-sterilized in 0.5% sodium hypochlorite, 0.05% Tween-80 for 10 min at RT and rinsed several times with sterile water. The leaves were then cut up with a sterile scalpel and placed on callus induction medium (CIM) agar for 1 week (22°C, 16 h light). The leaf/callus was then transferred to shoot induction medium (SIM) agar medium, and after 2–3 weeks successfully growing green callus was transferred to fresh SIM medium (either solid or liquid) and then maintained on this medium by regular subculture. CIM and SIM media were prepared as for the Gamborg's B-5 medium (above), except that the hormones (Sigma) differ: CIM, 1 mg l⁻¹ 2,4-dichlorophenoxyacetic acid, 0.2 mg l⁻¹ kinetin; SIM, 0.1 mg l⁻¹ NAA, 1 mg l⁻¹ 6-benzylaminopurine.

Isolation of the AtRAD50 cDNA and genomic DNA clones

Using poly(A)⁺ RNA prepared from the cell-suspension culture of *Arabidopsis*, the complete *AtRAD50* cDNA was isolated using the marathon RACE PCR kit following the manufacturer's instructions (Clontech).

The genomic DNA clone was isolated from a genomic DNA library constructed with Sau3A1 partially digested DNA from the cell suspension cloned into lambda FIXII (Stratagene Inc., La Jolla, CA, USA) following the manufacturer's protocol.

Southern analysis

Genomic DNA was isolated from cell-suspension cultures following the method of Dallaporta *et al.* (1983). DNA (3 µg) was digested with 50 units of the relevant restriction enzyme in a volume of 100 µl for 16 h at the recommended temperature.

Digested samples were phenol/chloroform extracted, ethanol precipitated, resuspended in TE and electrophoresed in 0.8% agarose/TAE gels. The gels were capillary blotted to Hybond N+ (Amersham, Orsay, France) positively charged nylon membrane, and hybridized at 62°C to radioactively labelled DNA probes according to Church and Gilbert (1984). Filters were then washed (0.1 × SSC, 0.1% SDS, 62°C) and autoradiographed. Probes were labelled with α -³²P dCTP using the Prime-It II kit (Stratagene) according to the manufacturer's instructions.

RNA isolation and Northern analysis

Frozen plant or cell-culture tissue was homogenized in liquid N₂ with a mortar and pestle. Total RNA was then prepared using the Trizol reagent following the manufacturer's instructions (Gibco-BRL, Cergy Pontoise, France). Poly(A)+ RNA was purified from total RNA using the mRNA Direct kit (DynaL Inc., Compiègne, France). For Northern analysis, 30 µg RNA per lane was fractionated on 0.8% agarose/formaldehyde gels, which were blotted, hybridized to radioactively labelled probes, and autoradiographed as for the Southern blots (above).

Callus MMS sensitivity tests

0.5 ml of suspension cells were pipetted onto the surface of agar plates containing solid SIM medium and different concentrations of MMS (Sigma #M4016). The plates were then incubated as described above, and resistance or sensitivity was scored visually 2-3 weeks later. MMS-containing plates were prepared immediately before use. All MMS-contaminated material was quenched after use by soaking in 10% w/v sodium thiosulphate.

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GenBank accession number AF168748.

U.S. Serial No. 09/538,396
Group Art Unit: 1638

APPENDIX D

APPENDIX D - motifs from Example 4, Pfam added to Appendix A (2/14/02)

Docket 1116E

Serial Number 09/538,396

!!AA_MULTIPLE_ALIGNMENT 1.0 PileUP

Symbol comparison table: genrundata:blosom62.cmp CompCheck: 1102

GapWeight: 8 GapLengthWeight: 2

MSF: 1380 Type: P February 11, 2002 15:38 Check: 219 ..

U63139aa Human Rad50
Z75311aa Human Rad50
NM022246aa Rat Rad50
U66887aa Mouse Rad50
AAD15407aa Arabidopsis Rad50
AF168748aa Arabidopsis Rad50
1116sid2 SEQ ID NO: 2 Maize Rad50
X14814aa Yeast Rad50
Z75312aa C. elegans Rad50

FORMATTING:

MOTIFS: Identified in Example 4 and Pfam analysis

Leucine zipper - Underlined, LZ noted in left margin

Nucleotide binding motif - Italicized & underlined, NT in left margin

Nuclear localization signal - boxed, NLS in left margin

Rad50 Zn-Hook - Double-underlined, ZN noted in left margin

1 50

U63139aa ----- R S L G G I D K D S
Z75311aa MLIFSVRDMF A S L G I D K D S
NM022246aa ----- R S L G I D K D S
U66887aa ----- R S L G I D K D S
AAD15407aa -----
AF168748aa -----
1116sid2 -----
X14814aa ----- Y S Q S N D E T E G M
Z75312aa ----- K F L E V G D D H D V H K D L S C S

51 100

U63139aa Y I F G M Q V A Q D V N E L
Z75311aa Y I F G M Q V A Q D V N E L
NM022246aa Y I F G M Q V A Q D V N E L
U66887aa Y I F G M Q V A Q D V N E L
AAD15407aa
AF168748aa
1116sid2
X14814aa Y T G V K I A A T M L N
Z75312aa T A N F V T G C Q K K Q N S T D R K R V D A S T E I D V K

NT

NLS

APPENDIX D - motifs from Example 4, Pfam added to Appendix A (2/14/02)
Docket 1116E
Serial Number 09/538,396

101 150
U63139aa AVQ MVC KRT G . RTK . S S I
Z75311aa AVQ MVC KRT G . RTK . S S I
NM022246aa LVQ MLC KRT G . RIK . S S I
U66887aa AVH MLC KRT G . RMK . S S I
AAD15407aa
AF168748aa
1116sid2
X14814aa VT NI L M KTTT GQ V . N ST T SL AQ
Z75312aa CTA RLV . SG AAAL E HT AIKY . D TVNT S VC PNTA L

151 200
U63139aa S N C S GK Q I T
Z75311aa S N C S GK Q I T
NM022246aa C N C S GK Q I T
U66887aa C N C S GK Q I T
AAD15407aa
AF168748aa
1116sid2
X14814aa LY P Y C L S N Q M N
Z75312aa KH P F KY C T S KE Q L V Q R

LZ, NLS

201 250
U63139aa Q RQT G K EYQ E K Y KQY K C E SKE AQLT EI
Z75311aa Q RQT G K EYQ E K Y KQY K C E SKE AQLT EI
NM022246aa Q RQT G K ECQTE K Y RQY K C E SKE AQLA EI
U66887aa Q RQT G K ECQTE K Y KQY K C E SKE AQLA OEI
AAD15407aa
AF168748aa
1116sid2
X14814aa S K MSV LL QS H KLD R K A L H L T I QYNEE
Z75312aa VL FKK Q HE SKQ LY E HVRDKL VA Q QEECE RISK R EET

LZ

251 300
U63139aa KSYENE DPL KNR KE HN SK M DNE KALD R KQ EKD S LEE
Z75311aa KSYENE DPL KNR KE HN SK M DNE KALD R KQ EKD S LEE
NM022246aa KAYENE EPL KNR KE HN SK M DNE KALD R KQ EKD S LEQ
U66887aa RSYE E EPL KNR KE HN SK M DNE KALE R KQ EKD S LEQ
AAD15407aa le ets qkv a h k mm k d s t fke qrqy
AF168748aa LE ETS QKV A H K MM K D S T FKE QRQY
1116sid2 CCGT L L G A LT H KL
X14814aa SE BSQ NEI TEKSDK PK NQ FC LSK NLKNT T SD VKRLSN
Z75312aa E KANG Q KEE R H H EDTLTS FKTEL ON KKL SLIRV

LZ

301 350
U63139aa K EKVFGQ Q N LYHNH QRT . REK R VDCH KLNKE R N
Z75311aa K EKVFGQ Q N LYHNH QRT . REK R VDCH KLNKE R N
NM022246aa K EKVFGQ Q N LYHNH QRT . REK R VDCQ KLNKE R N
U66887aa K EKVFGQ Q N LYHNH QRT . REK R VDCQ KLNKE R N
AAD15407aa
AF168748aa
1116sid2
X14814aa D IL L K P QNLL N SKV . MDKNN Q RD ET S SLKDRQ S Q
Z75312aa . . . PYFG KREIEE RGSEGRSYGE ER Q K G KNNQERQE

LZ

APPENDIX D - motifs from Example 4. Pfam added to Appendix A (2/14/02)
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351

U63139aa QEK LV Q LQ R QEHIRAL QSA QLE. DGFERG
 Z75311aa QEK LV Q LQ R QEHIRAL QSA QLE. DGFERG
 NM022246aa QER LV Q LQ R QEHIRAL QSA HLE. DGFERG
 U66887aa QEK LV Q LQ R QEHIRAL QSA HLE. DGFERG
 AAD15407aa nak nymL s t l n t q f f h y n s t
 AF168748aa NAK NYML S T L N T Q F F H Y N S T
 1116sid2 KQN TH H D K C H P K
 X14814aa SLNS IRRQ E E G K Y EKN NHL S L KEA Q H F G SN ENSDM
 Z75312aa QKK FENR S S K V I H C Q Y L R L EN R E L D. ... EHDADI

401

U63139aa ERQIK FHKL E QEGE.A KTANQ M F A ETLKQKE
 Z75311aa ERQIK FHKL E QEGE.A KTANQ M F A ETLKQKS
 NM022246aa ERQIK FHEL E QERE.A KTA Q L S T EALKQRE
 U66887aa ERQIK FHEL E QERE.A KTA Q L S T EALKQRE
 AAD15407aa t v g e m t a s t a d c d
 AF168748aa T V G E M T A S T A D C D
 1116sid2 N A S V K K LZ
 X14814aa ..QVNHEMSQ F F I S Q D L T D T D Q F A K Q L E T N L S L K S T V D S Q N
 Z75312aa D I E I D A I T. I E G M S D K A R M K N C A Q S N L R A Q A A T K E V E M K T

451

U63139aa RD K G G R I E L S T I ..KQNEL NVKY LQQL G SDRLE
 Z75311aa RD K G G R I E L S T I ..KQNEL NVKY LQQL G SDRLE
 NM022246aa RD K G G R M E L T I I ..KQTEL NVRN LQQL G SDRLE
 U66887aa RD K G G R T E L T I I ..KQSEL HVRS LQQL G SDRLE
 AAD15407aa a d k s a k r. d i k m s i e i f f t v d k g t
 AF168748aa A D K S A K R. D I K M S I E I F F T V L K Q T
 1116sid2 A S G Q. I S L K K A V K F N S R NLS
 X14814aa E Y N K K R S K L H D S E L A E K S F S L S T O S L N H L
 Z75312aa N E K V K K E E Q L F K I K Q S O N A T A G M L L K E E A L R K L A D P L

501

U63139aa QELIKAER S A E K N S N V E T L K M E I S L N K A D R T K D Q M Q
 Z75311aa QELIKAER S A E K N S N V E T L K M E I S L N K A D R T K D Q M Q
 NM022246aa QELTKAER S A E K N S S I E T L K E I L N K A D R N K D Q M Q
 U66887aa QELTKAER S A E K N S S I E T L K E M S L N K A D R S K D Q M Q
 AAD15407aa q k n s g k e h h t n
 AF168748aa Q K N S G K E H H T N
 1116sid2 H L A L G D I S R V L
 X14814aa .N L T Y K E K Q S W E S E N I I P K L N Q K B E N I I Q E K F Q D R I M K T
 Z75312aa N A L T E C K KS K L K Q D I L K K K C A E A K N A E K D S

551

U63139aa H H T T T Q E L T K D K A D K D Q R K K S R S E T S Y FN
 Z75311aa H H T T T Q E L T K D K A D K D Q R K K S R S E T S Y FN
 NM022246aa H H T T T Q E L T K D K T D K D Q R K K S R S E T S Y FN
 U66887aa H H T T T Q E L T K D K T D K D Q R K K S R S E T S Y FN
 AAD15407aa a g d1 t rd cg l p
 AF168748aa A G D S T E Q N L K H K K D C G L P
 1116sid2 R D S S K N K N F
 X14814aa Q L Y A I K N T K L E K T E K L Q N D S R Q V F L T O F
 Z75312aa K Q . T L S A R K M T A Y Q R I Y D N N W G G Q A P F P W T P S K T F H K L N D

600

APPENDIX D - motifs from Example 4, Pfam added to Appendix A (2/14/02)
Docket 11162
Serial Number 09/538,396

601 650
U63139aa QEDWLHS KS IQT D LAKLNK A S EQNKNHIN NE KKEEQ
Z75311aa QEDWLHS KS IQT D LAKLNK A S EQNKNHIN NE KKEEQ
NM022246aa QEDWLHS KS IQT D LAKLNK A S EQNKNHIN NE KKEEQ
U66887aa QEDWLHS KS IQT D LAKLNK A S EQNKNHIN NE KKEEQ
AAD15407aa V lrs d sl r k n ml m q vn ns fhn t
AF168748aa V LRS D SL R K N ML M Q VN NS FHN T
1116sid2 N FWP S O Q K F S R E T R
X14814aa Q ADLEMD FQ L IN QK NIATNNKKH ELDR Y N L Y NT E
Z75312aa I EEDLRD Q LNV Q STMQ H Y R Q EES

651 700
U63139aa S E K LFDVCGSQDF ES D RLKEEI KSS QRAMLA AT
Z75311aa S E K LFDVCGSQDF ES D RLKEEI KSS QRAMLA AT
NM022246aa S E K LFDVCGSQDF ES D RLKEDI KSS QRAMLA AT
U66887aa S E K LFDVCGSQDL ES G RLKEEI KSS QRAMLA AT
AAD15407aa k e v t a e s k d i e y m
AF168748aa k e v t a e s k d r e y m
1116sid2 L I A N M Q L E F LZ, ZN
X14814aa QDNQ. SK K V L . SENL PE CTIDEY V EET LSY TALENLK H
Z75312aa T QEL LS N IFE C . . C EA EVSEK E NLRK LKKA DLAPLSAKS

701 750
U63139aa A Y Q ITQL TD . ENQSC V Q V QT A L Q V SDL QSKLRL P
Z75311aa A Y Q ITQL TD . ENQSC V Q V QT A L Q A SDL QSKLRL P
NM022246aa A Y Q ITQL TD . ENQSC G Q V QT A L Q V SDL QSKLRL P
U66887aa A Y Q ITQL TD . ENQSC V Q V QT A L Q V SDL QSKLRL P
AAD15407aa q fe k r q . e s . a s . ka g h
AF168748aa Q FE K R Q . E S . A . AS KA G H
1116sid2 E L H L K . N V . P . D Q N A ZN
X14814aa QTTLE NRR LEIAERDSC Y L S K EN SFKSKL QEL TKTD NF
Z75312aa N YDSYI . . . EESKSSG L D KTK K IN S L ENMTL F PTE

751 800
U63139aa L TES LKK K KRRDE LG V P R Q I D K . . KE E LRNK VNR
Z75311aa L TES LKK K KRRDE LG V P R Q I D K . . KE E LRNK VNR
NM022246aa L TES LKK K KRRDE LG V P R Q I D K . . KE E LRNR SVNR
U66887aa L TES LKK K KRRDE LG V P R Q I D K . . KE E LRNR SVNR
AAD15407aa l e s t t . . i t e l t e
AF168748aa l e s t t . . i t e l t e
1116sid2 V . . T N L ZN, LZ
X14814aa LKD QNEK EY HS L EKHI T NSIN R DN Q C EKAKE
Z75312aa QEE EKLVR L K . . BE II EGO . NE QRIVKE KE VREKNRKL

801 850
U63139aa IQRLK DI QET GTM P E AKVC TD.VTI F QM KD EK
Z75311aa IQRLK DI QET GTM P E AKVC TD.VTI F QM KD EK
NM022246aa IQRLK DI QET GTM P E AKVC TD.VTI F QM KD EK
U66887aa IQRLK DI QET GTM P E AKVC TD.VTI F QM KD EK
AAD15407aa l g e l s q k a . l n a i f q v s y q
AF168748aa L G E L S Q K A . L N A I F Q V S Y Q
1116sid2 ES F H M . T T H V E Q Q V E LZ
X14814aa TKTSKSL E EVDS K K D L A E R L I K P T Y L E K KD ENS
Z75312aa MAEKS NLS KNEQ ET AKLKL A D . TDVG V QOL YEQTEENS

APPENDIX D - motifs from Example 4, Pfam added to Appendix A (2/14/02)
Docket 1116E
Serial Number 09/538,396

851 900
U63139aa AQQAARKQG IDLD. QQ QK EKH KL SSK LN KLIQDO
Z75311aa AQQAARKQG IDLD. QQ QK EKH KL SSK LN KLIQDO
NM022246aa AQQAARKQG VDLD. QQ QK EKH KL SSK LN KLIQDO
U66887aa AQQAARKQG VDLD. QQ QK EKH RL TSK LN KLIQDO
AAD15407aa K t r l s s s s k h g k d q i y e
AF168748aa K F R L S S S S K H G K d q i y e
1116sid2 A R L S S S S S K H G K d q i y e LZ
X14814aa SKT SEE SI YNTSEDG QT DELRDQQR MN RELRK T S LQMEKD
Z75312aa Y Q VSES DGLSY ... RKKVE D EYRK V QEG ELQKCS

901 950
U63139aa Q QHLKSTT NEL S QI ... N Q R Q L Q T E T VQS YR
Z75311aa Q QHLKSTT NEL S QI ... N Q R Q L Q T E T VQS YR
NM022246aa Q QHLKSTT NEL S QI ... N Q R Q L Q T E T VQS YR
U66887aa Q QHLKSTT NEL S QI ... N Q R Q L Q T E T VQS YR
AAD15407aa r c l a a v a ... n r d v t e r s d
AF168748aa R C L A A V A ... N R D V T E R S D
1116sid2 S A V N A ... E F L S LZ
X14814aa K RENS RMI NLI KE TV EIE S TQK NI S RSK R MIND DSR
Z75312aa R N K L S L NELGTH SL GEAA ... QA GAFAC ETK IKIQEC TA .

951 1000
U63139aa I DAK. Q S ETT KFO KEE I K NTSNKIAQ LN IKEK KN
Z75311aa I DAK. Q S ETT KFO KEE I K NTSNKIAQ LN IKEK KN
NM022246aa I DAK. Q N EIA KFO KEE I H K NTSNKMAQ IN IKEK KN
U66887aa I DAK. Q S ETA KFO KEE I H K HTSNKMAQ IN IKEK KN
AAD15407aa V Y T. G Q S D I R NQ EE
AF168748aa V Y T. G Q S D I R NQ EE
1116sid2 K L D S L E H LZ
X14814aa V E A R I S K N K E A Q S V L D K E R I Q V R K Q K T V A I R L R
Z75312aa I S Q K R N D P D A Q F K D E T R N V S K E E K K K A E M E Q M M L L K F H

1001 1050
U63139aa HGYMKD EN YIQDGR DY KQ ET K ... V I S E E K H K E N
Z75311aa HGYMKD EN YIQDGR DY KQ ET K ... V I S E E K H K E N
NM022246aa HGYMKD EN YIQDGR DY KQ ET K ... V V I E E D K H K E N
U66887aa HGYMKD EN YIQDGR DY KQ ET K ... V A V E E E K H K E N
AAD15407aa l a s y n d c f t r h l g q r s d e e k e
AF168748aa L A S Y N E ... H L G ... D E Q R S D E K E
1116sid2 G N M K ... S N L K H V C H M LZ
X14814aa F Q T Y N E V D F E A K G F E L Q T T I E L K ... L E L K E Q L D L K
Z75312aa R K S F K Q E ... G C E Q L M D E N N ... A T L N S E E N Q Q K F E

1051 1100
U63139aa E R L M Q D D T K I Q E W Q T L R N E K E E ... E R K Q H K E
Z75311aa E R L M Q D D T K I Q E W Q T L R N E K E E ... E R K Q H K E
NM022246aa K G T M Q D D T K I Q E W Q T L R N E K E E ... E R K Q H K E
U66887aa K G T M Q D D T K I Q E W Q T L R N E K E E ... E R K Q H K E
AAD15407aa g n d x n d n t k e q n
AF168748aa G N R N D N T K E Q N
1116sid2 A S Q Q G K Q R S LZ
X14814aa N V E E R K A D S M N E E K Q E L I E L Q Q H E S S R E ... Q N
Z75312aa E R S ... F D S S H R E S I K E T R M I E N K K E K T A F G O N E D

APPENDIX D - motifs from Example 4, Pfam added to Appendix A (2/14/02)
Docket 1116Z
Serial Number 09/538,396

| | | | |
|------------|----------------|----------------------|------------------------------------|
| | 1101 | | 1150 |
| U63139aa | Q QV QMK | HQ LEENI | N KRNH LAL RQKG EEE IHF K EP |
| Z75311aa | Q QV QMK | HQ LEENI | N KRNH LAL RQKG EEE IHF K EP |
| NM022246aa | Q QV QMKN | HQ LEENI | T KRNHSLAL RQKG EEE LHF K EP |
| U66887aa | Q QV QMKN | HQ LEENI | T KRNHSLAL RQKG EEE LHF K EP |
| AAD15407aa | S | .. | v ilr .. l .. cr .. e s .. n v .. |
| AF168748aa | G | .. | V ILR .. L .. CR .. E S .. N V .. |
| 1116sid2 | S | .. | KHS .. N .. F .. WQ .. H Q .. L .. |
| X14814aa | AEAERDKYQE | ESL LRTRE | LS NAGKL SE KQL N DSLTHO |
| Z75312aa | ...RIT QKQ | AYN LQ LRL | L GN EV IY TQ QE EK K KIAEAK STK |
| | | | LZ |
| 1151 | | | 1200 |
| U63139aa | A E R EMM V | V .. I | K .. Q |
| Z75311aa | A E R EMM V | V .. I | K .. Q |
| NM022246aa | A E R EMM V | V .. I | K .. Q |
| U66887aa | A E R EMM V | V .. I | K .. Q |
| AAD15407aa | hf d l | | |
| AF168748aa | HF D L | | |
| 1116sid2 | T N | | |
| X14814aa | D .. N H KE | E Q RS FVTD | V S K S .. G Q |
| Z75312aa | ECQNA SNR | DAI EAI K E IS | T AR NC A .. Q .. E GR G |
| | | | LZ |
| | 1201 | | 1250 |
| U63139aa | RS | E R | ADENV A SDKR N N LK A |
| Z75311aa | RS | E R | ADENV A SDKR N N LK A |
| NM022246aa | RS | E R | ADENV A SDKR N N LK A |
| U66887aa | RS | E R | ADENV A SDKR N N LK A |
| AAD15407aa | | h .. | |
| AF168748aa | | R .. | |
| 1116sid2 | | S .. | |
| X14814aa | D KR S T T K | R .. EV S TVKG | N YKQ V |
| Z75312aa | D RKV NST TT R | R ATSET S KKV YE NVN | HE .. |
| | | | |
| | 1251 | | 1300 |
| U63139aa | | | RE I |
| Z75311aa | | | RE I |
| NM022246aa | | | RE I |
| U66887aa | | | RE I |
| AAD15407aa | | | |
| AF168748aa | | | |
| 1116sid2 | | | |
| X14814aa | | | |
| Z75312aa | | | |
| | | | LZ, NT |
| | 1301 | | 1350 |
| U63139aa | H E K | SQ | R DVE RSEYV |
| Z75311aa | H E K | SQ | R DVE RSEYV |
| NM022246aa | H E K | SQ | R DVE RSEYV |
| U66887aa | H E K | SQ | R DVE RSEYV |
| AAD15407aa | G .. D | | |
| AF168748aa | G .. D | | |
| 1116sid2 | A .. | | |
| X14814aa | K HN NM | H .. | K LGH NAAAF H |
| Z75312aa | IV AD AE | PDENGKL RGRDM | LVNR TISCRP YI |

APPENDIX D - motifs from Example 4, Pfam added to Appendix A (2/14/02)
Docket 1116E
Serial Number 09/538,396

```

1351
U63139aa KENID C E VKCS SSL GFNVH-----
Z75311aa KENID C E VKCS SSL GFNVH-----
NM022246aa KENID C E VKSS NSL GSYVH-----
U66887aa KENMD C E VKCS SSL GSYVH-----
AAD15407aa KENMD C E VKCS SSL GSYVH-----
AF168748aa KENMD C E VKCS SSL GSYVH-----
1116sid2 KENMD C E VKCS SSL GSYVH-----
X14814aa KENMD C E VKCS SSL GSYVH-----
Z75312aa CLGMD HGI F SKRYPDGT VKRVMTKRRF

```



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Analysis Browser: [Level Up](#)

Report for 1116E.rad50.sid2 (Protein)

[Update](#)

Description 1116E_rad50_sid2

[Edit](#)

Function **DNA repair protein RAD50 (153 kDa protein).**

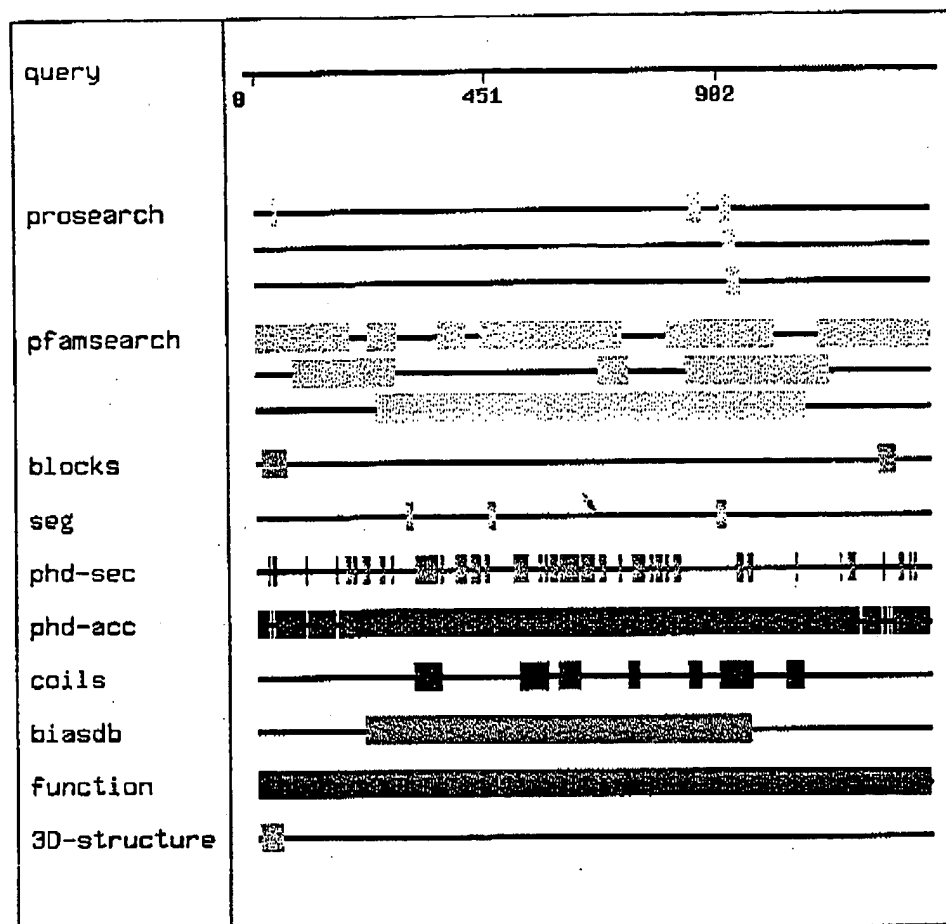
Direct assignment of functionality by homology to
swiss|P12753|RA50_YEAST

in region 1 to 1314 for overall length of 350 (99% of query, 375% of hit, [see the alignment](#)).

Functional class Transcription

Extracted keywords [ATP-binding](#), [Coiled coil](#)

Features
Summary

**Homologies****Protein**

30 clear homologs

All BLAST hits**ESTs**

170 homologs

All protein BLAST hits**Patents**

53 homologs

All EST BLAST hitsAll patent hits**General****Gene name****Molecular weight**

152.50 kD

Sequence length

1316

Isoelectric point

6.04

Predicted cellular localisation (PHD and PreLoc)nuclear (94.7 %)**3D Structure**

3D structure inferred by unlikely homology from residues 6 to 48 in 1E69-A

| | | |
|------------------|---|--|
| | View | alignment |
| | pdb 1E69 1E69-A | structure |
| Phylogeny | Distribution | 34 species extracted from 175 homologous sequences. Species |
| | Taxa | Chordata, Eukaryotae, Fungi, Planta |
| | Model organisms | <i>Arabidopsis thaliana</i> , <i>Caenorhabditis elegans</i> , <i>Drosophila melanogaster</i> , <i>Homo sapiens</i> , <i>Mus musculus</i> , <i>Saccharomyces cerevisiae</i> |
| Features | Coiled coil region | from 311 to 336, from 341 to 361, from 517 to 538, from 541 to 569, from 592 to 633, from 727 to 747, from 844 to 867, from 903 to 928, from 930 to 969, from 1036 to 1066 detected by [Coils] |
| | Low complexity region | from 296 to 307, from 456 to 469, from 902 to 915 detected by [seg] |
| | E-rich region | from 212 to 964 detected by [biasdb] |
| | No significant hits detected by | [Phd-tm] |
| Patterns | SMC family, C-terminal domain - region | from residue 1100 to 1314. Source: [pfamsearch] . Quality: (E=0.52) |
| | ABC transporter - region | from residue 1141 to 1296. Source: [pfamsearch] . Quality: (E=0.19) |
| | Intermediate filament protein - region | from residue 840 to 1118. Source: [pfamsearch] |
| | Myosin tail - region | from residue 237 to 1075. Source: [pfamsearch] |
| | Uncharacterized ACR, COG1579 - region | from residue 804 to 1015. Source: [pfamsearch] |
| | KE2 family protein - region | from residue 891 to 983. Source: [pfamsearch] |
| | Rad50 zinc hook motif region | from residue 673 to 726. Source: [pfamsearch] . Quality: (E=9.9e-06) |
| | Protein of unknown | from residue 441 to 717. Source: [pfamsearch] |

**function, DUF259 -
region**

**Late embryogenesis
abundant (LEA)
group - region** from residue 548 to 625. Source: [\[pfamsearch\]](#)

**Rad50 zinc hook
motif region** from residue 360 to 413. Source: [\[pfamsearch\]](#)

**Heat shock protein
9 / 12 - region** from residue 219 to 275. Source: [\[pfamsearch\]](#)

**Poly(A) polymerase
central domain -
region** from residue 75 to 273. Source: [\[pfamsearch\]](#)

**Sigma-70, non-
essential region -
region** from residue 112 to 260. Source: [\[pfamsearch\]](#)

**RecF/RecN/SMC N
terminal domain -
region** from residue 2 to 182. Source: [\[pfamsearch\]](#) . Quality:
(E=0.48)

**LEUCINE_ZIPPER
region** from residue 922 to 944. Source: [\[prosite\]](#)

from 915 to 937. Source: [\[prosite\]](#)

from 908 to 930. Source: [\[prosite\]](#)

from 849 to 871. Source: [\[prosite\]](#)

ATP_GTP_A region from residue 34 to 42. Source: [\[prosite\]](#)

**No significant hits
found in** [\[blocks database\]](#)

Comment No comment section.

[Edit](#)

Completed Tasks

Start Time User Comment Output

04.04.2003, dressvm bioSCOUT_default details...
16:44:19

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Alignment: 1116E.rad50.sid2 - pdb|1E69|1E69-A

BLASTP - alignment of 1116E.rad50.sid2 against pdb|1E69|1E69-A

chromosome segregation smc proteinfragment: smc fusion of the n- and c-terminal globular domains
residues 1-152 and 1023-1164;

- This hit is scoring at : 0.56 (expectation value)
- Alignment length (overlap) : 43
- Identities : 44 %
- Scoring matrix : BLOSUM62 (used to infer consensus pattern)
- Database searched : nrdb

```
Q:      6 KMLIKGIRSFDPDNKNVITFFKPLTLIVGPNAGKTTIIECLK      48
          K:::KG::SF.  .:::I F . :T.IVGPNAG:GK:.II:::K
H:      5 KLYLKGFKSFG--RPSLIGFSORVTAIVGPNAGSKSNIIDAIK      45
```

Legend of Alignment

- : positive score
- . score between -2 and 0

Please report problems and feedback concerning bioSCOUT through the [support interface](#).

Entry Page

HEADER CHROMOSOME SEGREGATION 09-AUG-00 1E69
TITLE SMC HEAD DOMAIN FROM THERMOTOGA MARITIMA
COMPND MOL_ID: 1;
COMPND 2 MOLECULE: CHROMOSOME SEGREGATION SMC PROTEIN;
COMPND 3 CHAIN: A, B, C, D, E, F;
COMPND 4 FRAGMENT: SMC FUSION OF THE N- AND C-TERMINAL GLOBULAR
COMPND 5 DOMAINS RESIDUES 1-152 and 1023-1164;
COMPND 6 ENGINEERED: YES
SOURCE MOL_ID: 1;
SOURCE 2 ORGANISM SCIENTIFIC: THERMOTOGA MARITIMA;
SOURCE 3 ATCC: DSM3109;
SOURCE 4 CELLULAR_LOCATION: CYTOSOL;
SOURCE 5 EXPRESSION_SYSTEM: ESCHERICHIA COLI;
SOURCE 6 EXPRESSION_SYSTEM_STRAIN: C41(DE3);
SOURCE 7 EXPRESSION_SYSTEM_CELLULAR_LOCATION: CYTOSOL;
SOURCE 8 EXPRESSION_SYSTEM_VECTOR_TYPE: PLASMID;
SOURCE 9 EXPRESSION_SYSTEM_PLASMID: PHIS17
KEYWDS SMC, STRUCTURAL MAINTENANCE OF CHROMOSOMES, COILED COIL
EXPDTA X-RAY DIFFRACTION
AUTHOR J.LOWE, S.C.CORDELL, F.VAN DEN ENT
REVDAT 2 27-MAR-01 1E69 1 JRNL
REVDAT 1 09-AUG-00 1E69 0
JRNL AUTH J.LOWE, S.C.CORDELL, F.VAN DEN ENT
JRNL TITL CRYSTAL STRUCTURE OF THE SMC HEAD DOMAIN: AN ABC
JRNL TITL 2 ATPASE WITH 900 RESIDUES ANTIPARALLEL COILED-COIL
JRNL TITL 3 INSERTED
JRNL REF J.MOL.BIOL. V. 306 25 2001
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Summary

Searched query 1116E.rad50.sid2 against PFAM database.

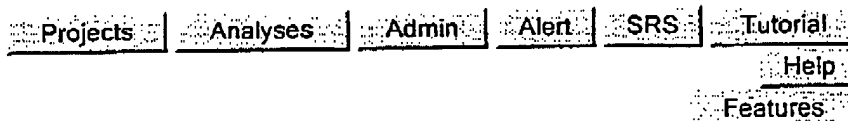
| Hit | Score | Expect | Description | Q from | Q to | Method |
|--|--------|---------|---|--------|------|---------|
| pfam hmm Rad50_zn_hook_alignment | 32.5 | 9.9e-06 | Rad50 zinc hook motif | 673 | 726 | HMMPFAM |
| pfam hmm Rad50_zn_hook_alignment | 4.2 | 1.8 | Rad50 zinc hook motif | 360 | 413 | HMMPFAM |
| pfam hmm HSP9_HSP12_alignment | -11.4 | 4.1 | Heat shock protein 9 / 12 - | 219 | 275 | HMMPFAM |
| pfam hmm LEA_1_alignment | -14.3 | 5.6 | Late embryogenesis abundant (LEA) group - | 548 | 625 | HMMPFAM |
| pfam hmm KE2_alignment | -19.0 | 5.6 | KE2 family protein - | 891 | 983 | HMMPFAM |
| pfam hmm PAP_central_alignment | -45.9 | 7.6 | Poly(A) polymerase central domain - | 75 | 273 | HMMPFAM |
| pfam hmm ABC_tran_alignment | -47.7 | 0.19 | ABC transporter - | 1141 | 1296 | HMMPFAM |
| pfam hmm SMC_N_alignment | -69.5 | 0.48 | RecF/RecN/SMC N terminal domain - | 2 | 182 | HMMPFAM |
| pfam hmm DUF164_alignment | -90.1 | 2.8 | Uncharacterized ACR, COG1579 - | 804 | 1015 | HMMPFAM |
| pfam hmm SMC_C_alignment | -116.7 | 0.52 | SMC family, C-terminal domain - | 1100 | 1314 | HMMPFAM |
| pfam hmm sigma70_per_alignment | -122.2 | 4.9 | Sigma-70, non-essential region - | 112 | 260 | HMMPFAM |

bioSCOUT 1.5.3 - 1116E.rad50.sid2

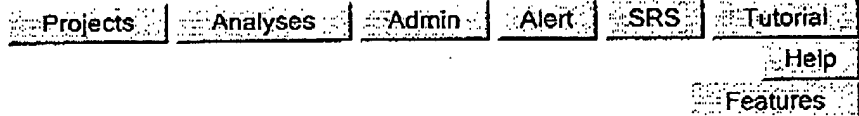
| | | | | | | |
|---|-------|-----|-----------------------|-----|------|---------|
| <input type="checkbox"/> pfam hmm DUF259 | - | 9.3 | Protein of unknown | 441 | 717 | HMMPFAM |
| alignment | 137.1 | | function, DUF259 - | | | |
| <input type="checkbox"/> pfam hmm filament | - | 5.4 | Intermediate filament | 840 | 1118 | HMMPFAM |
| alignment | 203.9 | | protein - | | | |
| <input type="checkbox"/> pfam hmm Myosin_tail | - | 9.8 | Myosin tail - | 237 | 1075 | HMMPFAM |
| alignment | 555.1 | | | | | |

| | |
|----------|------------------|
| New Task | Rename Sequences |
|----------|------------------|

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http://eshnc02.es.dupont.com:8101/cgi-bin/bioSCOUT/WWW/aliview?file=/LION/data/re... 5/21/2003
PAGE 44/64 * RCVD AT 11/26/2003 2:24:56 PM [Eastern Standard Time] * SVR:USPTO-EFXXF-1/4 * DNIS:8729307 * CSID:515 334 6883 * DURATION (mm-ss):23-16



Alignment: 1116E.rad50.sid2 - pfam|hmm|HSP9_HSP12

HMMPFAM - alignment of 1116E.rad50.sid2 against pfam|hmm|HSP9_HSP12

Heat shock protein 9 / 12 -

- This hit is scoring at : -11.4
- Scoring matrix : BLOSUM62 (used to infer consensus pattern)

```
Q: 219 --DQAHKLRENIAQDQEKSDASKSQMEQLKEKICGTEREILQMENSLDELRRLLQGQIDI 275
      D A K . A : : . . D : S K S . . E Q : K E K : . . . : . . TS D : . . : Q . D
H: 1 MSDlaRKdFgeKakEk1TPDSsKSTaEqvKEkvTDkLDkvAgkvtsdddKStvQkAhDk 59
```

Legend of Alignment

- : positive score
- . score between -2 and 0

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Alignment: 1116E.rad50.sid2 - pfam|hmm|LEA_1

HMMPFAM - alignment of 1116E.rad50.sid2 against pfam|hmm|LEA_1

Late embryogenesis abundant (LEA) group -

- This hit is scoring at : -14.3
- Scoring matrix : BLOSUM62 (used to infer consensus pattern)

```
Q: 548 LESSKDKLNEIVNEHKDKIKKVLrgrnpFEKDMKKEINQAFWVPSKEYNELRSKSQEAQ
   ::S:K:K:..... K:K:..... :D K.E .A . :KE. . R.K::EA:.
H: 1 MqSaKEKisnmAstAKekmditK.....AkadeKaEkatARTkeEkelAhqrkkAKEAgA
   ELKFTQSKVTDAREQLTK      625
   E:... ::K...A.E: ..
   eMdlheaKAehaaekesa      73
```

Legend of Alignment

- : positive score
 - . score between -2 and 0
-

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Alignment: 1116E.rad50.sid2 - pfam|hmm|KE2

HMMPFAM - alignment of 1116E.rad50.sid2 against pfam|hmm|KE2

KE2 family protein -

- This hit is scoring at : -19.0
- Scoring matrix : BLOSUM62 (used to infer consensus pattern)

```

Q: 891 ASSILERFQKSEELVLLAAEEKEQLIVEKKLLLEESLDPLSKEKE3-----LL-Q
   ...:L...Q: ...:L ...:K.QL : K .E L:.L.K .E. : Q
H: 1 vqellaklqqqlqqqlekvmqtqkaqlerqLkEaelvleELekldeDtkVYkLVGkvLVkvq

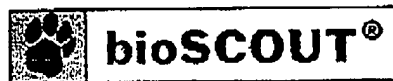
   EYNALKQKLDDEEYHQLAERKREFQQLDALGR----LNMKIKGYLDSKK 983
   : ...:L:E...QL.E. ....: : L : .L. K:: .L.S..
   dkceardeLeerleqleeeiktLekqeeylekeleeleeklqellqsaa 109

```

Legend of Alignment

- : positive score
 - . score between -2 and 0
-

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Alignment: 1116E.rad50.sid2 - pfam|hmm|PAP_central

HMMPFAM - alignment of 1116E.rad50.sid2 against pfam|hmm|PAP_central

Poly(A) polymcrase central domain -

- This hit is scoring at : -45.9
- Scoring matrix : BLOSUM62 (used to infer consensus pattern)

```
Q:      75      xSNWPLQDPSTLKKKFDDIFSAT-RYTKALEVIKK-LAKDQMQEIKTFRLK
          :P . PSTL :KF :FS. R: . . .V: K :.D.:E ..
H:      1 RFE.radP.lYPnavpstlvekfflvfsqWlrhnwpoPVlLkeinsdsieernlqrvrRF
          273
          E.rade      205
```

Legend of Alignment

- : positive score
 - . score between -2 and 0
-

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Alignment: 1116E.rad50.sid2 - pfam|hmm|ABC_tran

HMMPFAM - alignment of 1116E.rad50.sid2 against pfam|hmm|ABC_tran

ABC transporter -

- This hit is scoring at : -47.7
- Scoring matrix : BLOSUM62 (used to infer consensus pattern)

```
Q: 1141 -----TALDKALMRFS-----MKM-EEINKII-----KELWQ-----
      :L K: . . . . . : . . . . . : E :
H: 1 GevlalvGpNGaGKSTLLklisGllppteGtilldGardlsdlsklkerlerlrknigvv

      ----QTY---RGQDIDYISINSDS-----EGAGTRSY-----SYRVVMQTGDA--ELEM--
      . . . : I: . . . . . .R. : . . . : .G . .L R
      fQdptlfnpeltvreniafglrslglslskdeqrdrkkagaeelberlglydhldrr

      -GRCSAGQKVlasliiRLALAETFCNLGILALDEPTTNLDgpnAESIAAALLRIMEARK
      G..S.GQK      R:A:A.....:L.LDEPT..LD      .: .A.LL.....:
      pgtLSGGqkQ.....RvaiARAlltkpkllLlLDEPTagLD....pasraqlllellrelr

      GQeNFQLIVITHDERF-AHLIGQRQLAEKY      1296
      Q . . . . : ITHD . .L . . : .E.
      qq.ggTvllitHdlldlldrlaDrilvledG      199
```

Legend of Alignment

- : positive score
- . score between -2 and 0

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| | | | | | |
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| | | | | | Features |

Alignment: 1116E.rad50.sid2 - pfam|hmm|SMC_N

HMMPFAM - alignment of 1116E.rad50.sid2 against pfam|hmm|SMC_N

RecF/RecN/SMC N terminal domain -

- This hit is scoring at : -69.5
- Scoring matrix : BLOSUM62 (used to infer consensus pattern)

```

Q: 2 STVDKMLIKGIRSFdpdNKNVI-TFFKPLTLIVGPNAGAKTTIIECLKL---SCT-GEL
   . : : : : :G.:S: .K.VI .F . .T.IVGPNAG:GK:.I: : : : : : : :L
H: 1 mylkrielegFKSYa..gktvigpFspgFtaIvGPNAGSGKSNiLDAILFVLGegrsakkL

PPNSRSGHTEFVH--DPKVAGeteTKGQIKLRFKTAAGKDVV---CIRSFQLTQKASKME
... S. . . . . A. . . . . F... : : : : IR. .L: : : : :
RaerlsdLIhkgsggkppan...ksAeVtitFdnedkeniselgqhirdgpldeenpevt

FKAIESV---LQT-----I-----NFHTCEK---VCLSYRCADMREIPALMGVSKAV
... : : : I N . . K V ...A.:D E:..L... ..
ItRrvyrirlgldGstSeYyiWknrlrlNgkrvtklkevqelLesagIdiElatLangvsky

-----
RayawCFHWDkELiLleqIsWRaeYeaLapeiETCqlFLPElLtvSfqrGWeKetdYaEv
-----LENVIFVHQDE-----
. .... Q.E
LarnFERDkqLgYTqGPqKADLR)RANGLPVEDvLSRGQLKLknpYfiilOGeyltrekg

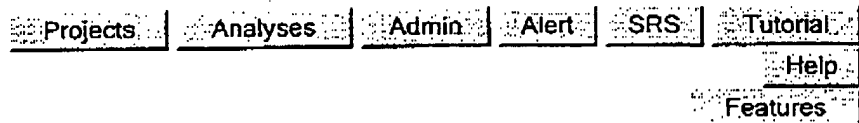
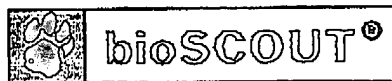
-----SNWPLQDPSTLK-----KKFDDIF-S 182
. : . . . P. . : : : : I :
rhClYLvedIasmkPkeRreldeGLlelleEEisGt 330

```

Legend of Alignment

- : positive score
- . score between -2 and 0

Please report problems and feedback concerning bioSCOUT through the [support interface](#).



Alignment: 1116E.rad50.sid2 - pfam|hmm|DUF164

HMMPFAM - alignment of 1116E.rad50.sid2 against pfam|hmm|DUF164

Uncharacterized ACR, COG1579 -

- This hit is scoring at : -90.1
- Scoring matrix : BLOSUM62 (used to infer consensus pattern)

```

Q: 804 PTDITDHFVHEIQQLVKEVEDLEYALDSSgrgVKSLEETIQLELNPLQRTROTLIVEVDDL
      .... : :IQ:: KE E LE .... K.L::: L.L::: .L E ::L
H: 1 mknekk.slvkiqidkekerLeerikei...pkelkkakellealkkeveeleqekeel

      RDQHRMLNEDMSSAQVRWHNAREEKVKA-----SSILERFQKSEBELVLLAEEKEQLI
      ::: :L:::..... : ..A EEK::: ...QK::: V.L.:E EQL
      keevkklekeiqeieekikka.EekmdeiktqrEYkALerElqakdkvltlrkeiegle

      VEKKLLEESLDPLSKEKESLLQEYNALKQKLDEEYHQLAERKREFQOELDalgRLNMKIK
      E K :EE:::L.:E . :E... :::: E....E: E. : : .L. K..
      eelkkieeieelkeeilkgEkeleeeeeevelEvrkikekvlellskre...elkekted

      GYLDSEKNEKLKELQGRHVL-----CH----SQLQSCMAKQ-QRIS-----
      I S .. :K. :. : : CH SQ:: :K: . I
      edllsfYERiiknknlvivPiennvCaGChiilpsqfenkVrkePddivfCPyCSRILY

      AEL      1015
      E
      yee      235
  
```

Legend of Alignment

- : positive score
- . score between -2 and 0

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Alignment: 1116E.rad50.sid2 - pfam|hmm|SMC_C

HMMPFAM - alignment of 1116E.rad50.sid2 against pfam|hmm|SMC_C

SMC family, C-terminal domain -

- This hit is scoring at : -116.7
- Scoring matrix : BLOSUM62 (used to infer consensus pattern)

```

Q: 1100 ISKHKQELKLS-----QYKDIEKRYTNQFLQKTTETANKDLDRYYTALDKAL
      :.K.:EL          :Y.:E:RY...:L...E..K.L.....LDK.
H: 1 lekeikeLgpVlnElqvNlkAieEYaeveeRyselvekledleeerkkllewieeldkkr

      MRFHSMKMEIEINKIIEKELWQQ-TyrgqdidyisinsdsegagtrsysyrvmQTGDAELE
      :. . . .:INK .KE::Q: T                      GDAEL.
      leeFmeafnkinknfkevFqeLt.....gGGdAeLr

      MRG---RCSAGQKVLAS-----LIIRL-----ALAETFCLNCG----ILALDEPT
      :.. S:G::A.      :.L      ALA .F.:      ...LDE .
      LtDpdDPFssGieisArPPgKkwkneleLSGGEKtLtALALlFAIhkykPsPFYvlDEVd

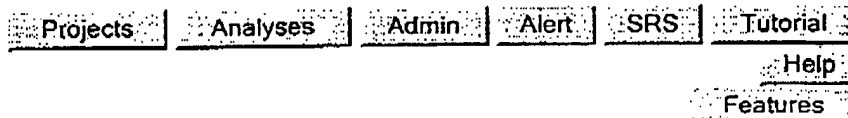
      TNLDGPNAESLAAALLRimearKGQENFQLIVITHDERFAhligqrQLAEKYYRVSKDEN
      . LD .N. S .A .: . R: .:N Q.IVI: . . . . : A:.. V .:..
      AALDeaNV.sRvAnyIk...rersknaQFIVIsLRnnmm.....ekADaLvGVymqdd

      QHSIIESQEI      1314
      S : S ::
      gvskVislKL      210
  
```

Legend of Alignment

- : positive score
- . score between -2 and 0

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Alignment: 1116E.rad50.sid2 - pfam|hmm|sigma70_ner

HMMPFAM - alignment of 1116E.rad50.sid2 against pfam|hmm|sigma70_ner

Sigma-70, non-essential region -

- This hit is scoring at : -122.2
- Scoring matrix : BLOSUM62 (used to infer consensus pattern)

```

Q: 112 ---AIESVLQITNPHTGEKVCLSYR---CADMDREIPALMG---SVSKAVLENVIFV---
   : : : L : : : E : LS : D D I : : : L : :
H: 1 fPgtdvyILaeYdRvetEagRLsDilsGyiDPddgiapdeAptathieselaeepssekD
   -----HQDESN-----WPlqDPSTLKKKFDDIfsATRYTKALEVIK--
   ..D:S. P DP.....F.: : : :K. : :K
   daadaddddDEdEeeessdddsEagdgpp..DPEeArerFgel..reqlektkkalkKh
   -----KLHKDQMQEI---KTFRLLKL---ENL-QTVKDQAHKLREN-----
   K : : : : : : : : L : L : V : : : : R :
   GRgskqalealeaLAelFmpikLvPKQfDaLVerVrgmldrvRkqERaIMklCVrdArMP
   -----IAQ-----DQEKSDASKS--QMEQLKEKICGTEREILQME 260
   I.. : KS... : : :E::KE.I. : : : : :E
   RkdFiksFpgnETnleWlekllkskkkyadeaLervkedilrcQqKLadIE 227

```

Legend of Alignment

- : positive score
- . score between -2 and 0

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Alignment: 1116E.rad50.sid2 - pfam|hmm|DUF259

HMMPFAM - alignment of 1116E.rad50.sid2 against pfam|hmm|DUF259

Protein of unknown function, DUF259 -

- This hit is scoring at : -137.1
- Scoring matrix : BLOSUM62 (used to infer consensus pattern)

```

Q: 441 DGQIQSKIESMSGILRRRKDKKERDAAEVELSK-----FNLSRID---
      .....G .....:R...:V:... F::D
H: 1 adamRamLDqLMGRdasnRngdesrqkkvkfdDpeVCrsyLvgfCPHDlFintKmDnLG
      -----ERERHMQIEVERKTLALG---ERDYDSIISQ-----KRTEVYSLEQKIKVLLR
      : : ....E ..K: - ERD ..... : ..E::L::KIK :
pCpkvHdlklradYErasksrdyfpkfErdaleflersvsEvtqsPEiLELEkikEkmk

EKDiinrnADERVKLGLKKDALESSKDKLNEIVNEHK--DKIKKVLGR-----
E. : D : ..K..LE..... : . E.K : . L .
EAFv....hDcdrridkakqrLeetqeeqtkaaaekRqaeeaeldeekAsLPqPvPAq

-----NPFEKDM-----KKEINQAFWPVdKEYNELRSKSQAEQ---E
      ..... : : : :A : KE..EL::K::E:E: E
PPssELPPDPPrTqEvIgklLaEaLGeeGkVdeaqklm.kevEeLkakkkeleeklde

LKFTQSKVTDAreqltklrrdmdakrrfldsklqsilqisanvdmfPKVLQDAMNKR---
:: .....A D.M.:
vrnaapssaqa.....WslDeMgqgKLR

-----DEQKRLNFANG-----MREMLAPFEHLARK-----NHVCPCCER
      D...RL...G :RE.LA.....K ... ER
VCEvCGAyLsvLDadrRIADHfgGKlHLGYvkiReklaeLkkakaklkeevkktgrekR

AFTPDEEDEFVKKQRMQ 717
...E:::..QR..
eereretektldgqRqh 335

```

Legend of Alignment

- : positive score
- . score between -2 and 0

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Alignment: 1116E.rad50.sid2 - pfam|hmm|filament

HMMPFAM - alignment of 1116E.rad50.sid2 against pfam|hmm|filament

Intermediate filament protein -

- This hit is scoring at : -203.9
- Scoring matrix : BLOSUM62 (used to infer consensus pattern)

```

Q:      840 ---EEIQLELN-----FLQRTDRLIVEVDDLRLDQH---R-MLNEDMSSAQ
      E::Q .LN          FL::...L V:::LR...  . . .: .:
H:      1 nEKqmQ.nLNDRLAsYIdKVRfLEqqNkeLevkielelrqkqsrqgpasvsrlyslYate

VRwhNAREEKVKASSILERFQKSEEEVLVLAEE-KEQLIV-----EKKL--L
. . .R.: .: . .R.Q . :L .E: .: .E .L
ie..eLRrqidqltnerarlqlEidnlrealedfrkKyedKeDLaaQnqlkdlEialntk

EESLDPLSKEKESLLQEYNAL-----KQKLDEEY-----HQLAE
E..L . . E:: . . .L . . .LDE. . .LA
eaeLaTaL.eRgeaEndlvGLRaQiAkLEslaaRkdLDeaTLarvDLEnkvEsLqEELaF

RKREFQQELDAL-----GR--LNMKIKGYL----DSKKNEKLKELQgrhvlchSQLQsCM
K:::E::L . . :N:: . . .L:E: . :Q: . :
LKknHeEEvkeLqaqiqdtgqvnVEmDaarqqEwkLDLtkalrEiR.....aQYE.ei

AKQQRISAEINKSKEELLQGQQLKRNIDNlkYRKTKADVEQLTRDIESLEERLLSigsL
A::R .AE .:L : Q ...RN : .R..K:::L.R.T:SLE .L S:
AeknrgeaEewYksKleeLqtaaarngae...lrakeEitElRRqiQsLeiELQsl..k

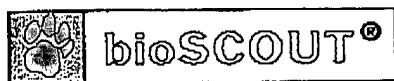
SAIEADLKRHSQEKERLNSEFNRWGTLsVYQSNISKHKQELK--LSQYKDI-----
S. . : : :ER::E. :Q::S .: .: .:E: .L::Y::
sqnasLErqiaElEeryeaelaqygalisqlEeeLqqlreEMarqLrEYQeLLdVKlaLD

-----EKR      1118
      E.R
iEiATYRKLLGEESR      359
    
```

Legend of Alignment

- : positive score
- . score between -2 and 0

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Alignment: 1116E.rad50.sid2 - pfam|hmm|Myosin_tail

HMMPFAM - alignment of 1116E.rad50.sid2 against pfam|hmm|Myosin_tail

Myosin tail -

- o This hit is scoring at : -555.1
- o Scoring matrix : BLOSUM62 (used to infer consensus pattern)

```
Q: 237 ASKSOMEQLKEKICGTEREILQMETSLOE-----LRRLOQCI----DIKATERS
      :.Q.:L:E: .E.E: Q:..L: : :L:..I : ..ER:
H: 1 dlerqkrelaeqlkrkeselsqlslklEdEqalvaqLqkkikeleaRIeEleSeLEaERA

T-----LLTQQHEKLAALSEENEDT-----DEELM-----EWQTKFEE
      . : :L..LSE. E: : :EL. E : :EE
ARaKaEkqRaDLsrELEeLsERLeEagGaTaaQiElnkKREaELaKLRrdLEEanlqhEe

RIALLETKISKLVDRMDDE----ASYSSVLKQNSSELTHEIGKLOAEADAHLTMKHERDS
      :.A.L..K : : :D: : : :K:..S:L. E:..L A:..D: : :K : :
alatLRKKHqdainElldQieqLqKqKakaEKeKsqlqaEvddllaqlsdtKaKlnaEK

DIKNICTKHNLGFPVPhpfTNDVAMNLT--NRIKARLSSLENDLLDKKKSNEQDLVLWK
      .K: : : : .V. : : :L. : : K:RL.S : :DL. : : E Q:.. L K
kakqlEsQlsElqvK...ldElqRqlnDltsqKsRLqsENsdLtrqleEaEaqvsqLsk

HYLKINA-----RYSEVDGQIQSKIESMSGILRRRKDK-----EKERDA---AEVELSK
      : : : R E : : : : : L. D. : : E:E:..A : E :ISK
lKsqlesQLEeAkRsleEEsReRanLqaqlrnlehDlDslrEqleEEsEAKaeleRqLsK

FNISRIDERERHMQIEVERKTLALGE--RDYDSIISOKRTEVYSLEQKIKVLLREKDIIN
      N :.I: : : : E : : .L E : : : IS: : : :K.. L :.K. :.
an.aeiqqwrsKfEsEgalraEELEEIKKlInqkisElEeaaEaanaKcssLEKtKsRLq

RNAD----ERVKLGLKKDALESSKDKLNEIVNEHKDKIKKVLGRNPFEEKDMKKEINQAF
      : : : E : : : : :LE: : : :I: E K.K: : : . : : : : :F
sElEDlqievEranaaaseLEKKqknFDKilaEwKkkvdelqaEletAqreaRnlstElf

WpVDKEYNELRSKSQEAQELKFTQSKVTDAREQLTKLRR---DMDAKRRFLDSKLQSI
      :..E..EL: : : : :E K .Q:..D:..QL : R : : : :RR L: : : :
r.lKneleElkDqvEaLrRENKnLqdEikDLtdqLgEgGRnvHELEKarRrLEaEkdeLq

QisANVDMfpkvLQDAMnKRDEQKRLNENFANGMREMLAPFE--HLARKnhvcpceraftp
      . A : : : A: : : :E.K L. : : : : :E .LA.K
a..ALeE.....AEaAL.eqeEsKvlRaqvE.lsqiRsEiERRLaEK.....

DEEDEfVKKQRMQnsstaerskalAMESSNAEaLFQQLDKLRTIYDAYVKLVEETIP---
      :EE E :.K.: : : A:ES .A. L :. K :. : . K :E .I
EEEfEntRKnhqr.....aiesLqas.LaEaEaKgKaEalRlKKKLEgdInELE

LAEKNLNQHLaDESQKAQAFDDLL-GVLAHVQMDRDAVEALLQPTDTIDRHVHEIQQLvk
      :A :.N: A: : : : : : : :.V: : : A E . : : :R.. :Q.
```

```
iaLDhankanaeaqKnvKkyqqqvkeLQtqvEeeQRaredareqlavaERRataLqa...
EVEDLEYALDSSGRGVKSLEaiqLELNFLQRTDRTLIVEVDDLDRDQHRMLNEDMSSAQVR
E:E:L..AL:: R. K..E .EL .... :.L... :.L Q.R.L. ::::Q
ELEELrvaLeqaeRaRKqAE...tElaEaservneLtaqnssLiaqKRKLEgelaalqsD
WHNAREEKVKAssiLERFQKSEEEELVLLAEEkeqLIVEKKLLLeESLDPLSKEKESLLQEY
..A .E .A ER :K:::.. LAEE L E:: :.L: L.K: ES ::E
LDEavnElkaA...eERakkaqaDaarLaeE...LrqEQehs.qklErIRKqLEsqvKe.
naLKQKLDE-EYHQLAE-----RKREFQQELDALGRNLNMKIKGYLdSKKNEKLKE
L: :LDE E .L. R RE...ELD. R :::: L .K...:KE
..LqvRLdEaEaaAlkgGKkvIqKLEaRVReLEaELdgEqRRhaetqKnL.RKaeRrvKE
LQG-----RHVLchsQLQSCMAKQQrisaelNKSKeLlqqgqQLKRNIDDNLKYRKTK-
LQ ::: :LQ... K Q K K ..KR::: ::::
LqfQvEEDkKnl...rlQDLvDKLq.....aKiK.....tyKRQLEeEaEEiaqinl
ADVEQLTRDIESLEERL-LSIGSLSAIE---ADLKRH 1075
:.....R::E..EER. : .SL::: A. :R.
sKyRkaQreLEdAEERADqAEsslnklRgreaKsRrs 864
```

Legend of Alignment

- : positive score
- . score between -2 and 0

Please report problems and feedback concerning bioSCOUT through the [support interface](#).

SeqWeb Sequence Analysis

HmmerPfam Results

Refine

Query: 1116SID2 from: 1 to: 1316 WPDEF Case 1116 Rad50 SEQ ID NO: 2

Scores for sequence family classification (score includes all domains):

| Model value | N | Description | Score | E- |
|----------------------|-------|---|--------|-------|
| ----- | ----- | ----- | ----- | ----- |
| <u>Rad50_zn_hook</u> | | Rad50 zinc hook motif | 36.3 | 6.9e- |
| 07 | 2 | | | |
| <u>HSP9_HSP12</u> | | Heat shock protein 9 / 12 | -11.4 | |
| 4.1 | 1 | | | |
| <u>LEA_1</u> | | Late embryogenesis abundant (LEA) group | -14.3 | |
| 5.6 | 1 | | | |
| <u>Histone_HNS</u> | | H-NS histone family | -28.3 | |
| 7.9 | 1 | | | |
| <u>ABC_tran</u> | | ABC transporter | -46.1 | |
| 0.14 | 1 | | | |
| <u>SMC_N</u> | | RecF/RecN/SMC N terminal domain | -69.5 | |
| 0.75 | 1 | | | |
| <u>TACC</u> | | Transforming acidic coiled-coil-contain | - | |
| 88.9 | 8 | 1 | | |
| <u>DUF164</u> | | Uncharacterized ACR, COG1579 | -90.1 | |
| 4.1 | 1 | | | |
| <u>Tropomyosin</u> | | Tropomyosin | - | |
| 91.4 | 8 | 1 | | |
| <u>SMC_C</u> | | SMC family, C-terminal domain | -116.7 | |
| 0.36 | 1 | | | |
| <u>sigma70_ner</u> | | Sigma-70, non-essential region | -122.2 | |
| 8.3 | 1 | | | |
| <u>filament</u> | | Intermediate filament protein | -203.8 | |
| 3.3 | 1 | | | |

http://cshpc03.cs.dupont.com:8000/gcg-bin/result.cgi/1116sid2_humncrp_543863.htm?project=Dress110702&nonav=1 (1 of 2) 11/25/2003 7:10:07 AM

PAGE 58/64 * RCVD AT 11/26/2003 2:24:56 PM [Eastern Standard Time] * SVR:USPTO-EFXXRF-1/4 * DNIS:8729307 * CSID:515 334 6883 * DURATION (mm:ss):23-16

SeqWeb Sequence Analysis

ERM Ezrin/radixin/moesin family -236.2
9.5 1

Parsed for domains:

| Model | Domain | seq-f | seq-t | hmm-f | hmm-t | score | E-value |
|---------------|--------|-------|-------|-------|-------|-------|---------|
| Rad50_zn_hook | 2/2 | 673 | 726 | 1 | 57 | 32.5 | 9.9e-06 |

Alignments of top-scoring domains:

Rad50_zn_hook: domain 2 of 2, from 673 to 726: score 32.5, E = 9.9e-06

```
*->galesekaelkkaieeleeeesscCPvCgReLgteeeekkelikeyks
+ ++++++l ++ +++ + CP+C+R + t++e+ e++k+++
1116SID2 673 NFANGMREMLAPFEHLARKNHV--CPCCERAF-TPDEEDEFVKKQRM
716
```

```
eldrlpeelk<-*
+ +++ e k
1116SID2 717 QNSSTAERSK 726
```

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Pfam: Rad50_zn_hook

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Rad50_zn_hook motif




Figure 1: 118d Replication
Rad50 coiled-coil zn hook

Accession number: PF04423

Rad50 zinc hook motif

The Mre11 complex (Mre11 Rad50 Nbs1) is central to chromosomal maintenance and functions in homologous recombination, telomere maintenance and sister chromatid association. The Rad50 coiled-coil region contains a dimer interface at the apex of the coiled coils in which pairs of conserved Cys-X-Cys motifs form interlocking hooks that bind one Zn ion. This alignment includes the zinc hook motif and a short stretch of coiled-coil on either side.

INTERPRO description (entry IPR007517)

The Mre11 complex (Mre11 Rad50 Nbs1) is central to chromosomal maintenance and functions in homologous recombination, telomere maintenance and sister chromatid association. The Rad50 coiled-coil region contains a dimer interface at the apex of the coiled coils in which pairs of conserved Cys-X-Cys motifs form interlocking hooks that bind one Zn ion. This alignment includes the zinc hook motif and a short stretch of coiled-coil on either side.

| Domain | Chain | Start Residue | End Residue |
|---------------|-------|---------------|-------------|
| Rad50_zn_hook | A | 421 | 475 |
| Rad50_zn_hook | B | 421 | 475 |

The Swissprot/PDB mapping was provided by **MSD**

| Alignment | Domain organisation |
|-----------|---------------------|
| | |

http://www.sanger.ac.uk/cgi-bin/Pfam/getacc/PF04423 (1 of 3) 11/25/2003 6:43:22 AM

Pfam: Rad50_zn_hook

| | |
|--|---|
| <input checked="" type="radio"/> Seed (17) <input type="radio"/> Full (25) Format Coloured alignment Get alignment View HMM logo Further alignment options here Help relating to Pfam alignments here | <input checked="" type="radio"/> Seed (17) <input type="radio"/> Full (25) As a Graphic Zoom <input type="text" value="0.5"/> pixels/aa. View Graphic As a Tree <input type="checkbox"/> Bootstrap tree NIFAS Applet To find out about the NIFAS tree-viewer, click here |
| Species Distribution | |
| NEW! View alignments & domain organisation by species Tree depth: <input type="text" value="Show all levels"/> View Species Tree | |
| Phylogenetic tree | |
| <input checked="" type="radio"/> Seed (17) <input type="radio"/> Full (25) Download tree ATV Applet The trees were generated using Quicktree To find out more about ATV phylogenetic tree-viewer click here | |

Database References

| | |
|--|--|
| PDB You can find out how to set up Rasmol here | 118d A: 421; 475; PDB-2: Pfam1 CATH:PD551M MSD Scop[Cath: Pfam1] Scop-UK Rasmol (html) Scop-USA Cntrl (ps) |
| SYSTEMS | Rad50 zn_hook |
| PANDIT | Rad50 zn_hook |

Pfam: Rad50_zn_hook

Literature References

1. Teihering on the brink: the evolutionarily conserved Mre11-Rad50 complex.
Connelly JC, Leach DR;
Trends Biochem Sci 2002;27:410-418.
2. The Rad50 zinc-hook is a structure joining Mre11 complexes in DNA recombination and repair.
Hopfer KP, Craig L, Moncalian G, Zinkel RA, Usui T, Owen BA, Karcher A, Henderson B, Bodmer JL, McMurray CT, Carmey JP, Petrini JH, Talner JA;
Nature 2002;418:562-566.

Pfam specific information

| | |
|--------------------------|-----------|
| Author of entry | Bateman A |
| Type definition | Motif |
| Alignment method of seed | Clustalw |
| Source of seed members | Bateman A |
| Average Length | 55.6 |
| Average %id | 24 |
| Average Coverage | 5.34% |


HMMER build information


| | Pfam_Is [Download HMM] | Pfam_fs [Download HMM] |
|---------------------|---|--|
| Gathering cutoff | 25.0 25.0; | 25.0 25.0 |
| Trusted cutoff | 40.9 40.9; | 31.4 38.9 |
| Noise cutoff | 22.6 22.6; | 24.6 20.6 |
| Build method of HMM | hmmbuild -F HMM_Is SEED hmmcalibrate --seed 0 HMM_Is | hmmbuild -f -F HMM_fs SEED hmmcalibrate --seed 0 HMM_fs |

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Pfam: Mre11_DNA_bind

 **Protein families database of alignments and HMMs**



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Mre11_DNA_bind

Accession number: PF04152

Previous identifiers: Mer11_DNA_bind;

Mre11 DNA-binding presumed domain[Add Annotation](#)

The Mre11 complex is a multi-subunit nuclease that is composed of Mre11, Rad50 and Nbs1/Xrs2, and is involved in checkpoint signalling and DNA replication [1]. Mre11 has an intrinsic DNA-binding activity that is stimulated by Rad50 on its own or in combination with Nbs1 [2].

INTERPRO description (entry IPR007281)

The Mre11 complex is a multi-subunit nuclease that is composed of Mre11, Rad50 and Nbs1/Xrs2, and is involved in checkpoint signalling and DNA replication [MEDLINE:21984524]. Mre11 has an intrinsic DNA-binding activity that is stimulated by Rad50 on its own or in combination with Nbs1 [MEDLINE:20300914].

| Alignment | Domain organisation |
|--|--|
| <input checked="" type="radio"/> Seed (10) <input type="radio"/> Full (20) | <input checked="" type="radio"/> Seed (10) <input type="radio"/> Full (20) |
| Format: <input type="text" value="Coloured alignment"/> | <div>As a GraphicAs a Tree</div> |
| <input type="text" value="Get alignment"/> | Zoom <input type="text" value="0.5"/> pixels/aa. <input type="checkbox"/> Bootstrap tree |
| <input type="text" value="View HMM logo"/> | <input type="text" value="View Graphic"/> <input type="text" value="NIFAS Applet"/> |
| Further alignment options here | To find out about the NIFAS tree-viewer, click here |
| Help relating to Pfam alignments here | |
| Species Distribution | Phylogenetic tree |
| NEW! View alignments & domain organisation by species | <input checked="" type="radio"/> Seed (10) <input type="radio"/> Full (20) |
| Tree depth: <input type="text" value="Show all levels"/> | <input type="text" value="Download tree"/> <input type="text" value="ATV Applet"/> |
| <input type="text" value="View Species Tree"/> | The trees were generated using Quicktree |
| | To find out more about ATV phylogenetic tree-viewer click here |

Database References**SYSTEMS**[Mre11_DNA_bind](#)

Pfam: Mre11_DNA_bind

PANDIT

Mre11_DNA_bind

| Literature References |
|--|
| 1. <u>A mechanistic basis for Mre11-directed DNA joining at microhomologies.</u> Pauli TT, Gellert M; Proc Natl Acad Sci U S A 2000;97:6409-6414. |
| 2. <u>The Mre11 complex: at the crossroads of dna repair and checkpoint signalling.</u> D'Amours D, Jackson SP; Nat Rev Mol Cell Biol 2002;3:317-327. |

| Pfam specific information | |
|---------------------------|----------------------------|
| Author of entry | Wood V, Finn RD |
| Type definition | Domain |
| Alignment method of seed | Clustalw |
| Source of seed members | Pfam-B_3909 (release 7.3); |
| Average Length | 200.3 |
| Average %id | 39 |
| Average Coverage | 28.59% |

| HMMER build information | | |
|-------------------------|---|--|
| | Pfam_ls [Download HMM] | Pfam_fs [Download HMM] |
| Gathering cutoff | 25.0 25.0; | 25.0 25.0 |
| Trusted cutoff | 71.9 71.9; | 49.5 32.0 |
| Noise cutoff | 13.1 13.1; | 8.2 17.4 |
| Build method of HMM | hmmbuild -F HMM_ls SEED hmmcalibrate --seed 0 HMM_ls | hmmbuild -f -F HMM_fs SEED hmmcalibrate --seed 0 HMM_fs |

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